POTENCIALIDADE DE CORRECTIVOS ORGÂNICOS/INORGÂNICOS NA RECUPERAÇÃO DE ESCOMBREIRAS, DE *GOSSAN* E RICAS EM SULFURETOS, E DESENVOLVIMENTO DE *CISTUS LADANIFER* E *LAVANDULA PEDUNCULATA* PARA A EXPLORAÇÃO DE BIOEXTRACTOS VEGETAIS

ERIKA DA SILVA DOS SANTOS

ORIENTADOR: Professora catedrática Maria Manuela Silva Nunes Reis Abreu
COORIENTADOR: Professor catedrático Felipe Macías Vázquez (Universidad de Santiago de Compostela)

TESE ELABORADA PARA OBTENÇÃO DO GRAU DE DOUTOR EM ENGENHARIA DO AMBIENTE

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RESUMO

Neste trabalho estudou-se a potencial reabilitação de dois tipos de escombreiras, rica em sulfuretos e *gossan*, da mina de São Domingos usando resíduos orgânicos/inorgânicos e espécies vegetais com valor económico, *Cistus ladanifer* (esteva) *e Lavandula pedunculata* (rosmaninho). Avaliou-se o comportamento ecofisiológico das espécies em condições naturais e a qualidade de alguns produtos derivados das mesmas (chá/infusão de rosmaninho e bioextractos de ambas as espécies) face às características dos substratos onde estas crescem espontaneamente. Em condições controladas, avaliou-se a eficiência de Tecnossolos, elaborados com resíduos orgânicos/inorgânicos e de escombreiras, na melhoria da qualidade química dos lixiviados (percolação e simulados), para minimizar a dispersão dos contaminantes, e melhorar as características químicas e biológicas dos materiais para desenvolvimento de vegetação.

Independentemente das características dos solos, ambas as espécies crescem sem sinais de toxicidade ou deficiência nutricional apresentando características ecofisiológicas adequadas para fitoestabilização. Nas duas espécies, o primeiro mecanismo de tolerância aos elementos potencialmente tóxicos (EPTs) do solo é a sua acumulação na raiz e baixa translocação para locais fotossinteticamente activos. As concentrações fitotóxicas de alguns elementos na parte aérea do rosmaninho não afectaram a concentração de pigmentos e proteínas nem desencadearam mecanismos de tolerância, enzimáticos e não enzimáticos, relacionados com stresse oxidativo. Os produtos derivados das plantas crescendo na área mineira apresentaram viabilidade económica para diferentes usos pois mantêm a sua qualidade comparada com as de áreas controlo.

As escombreiras ricas em sulfuretos lixiviaram soluções extremamente ácidas e com maiores concentrações de EPTs do que as escombreiras de *gossan*. A metodologia mais sustentável para a reabilitação de ambas as escombreiras da mina de São Domingos foi o desenvolvimento de um sistema que combina o isolamento natural dos materiais ricos em sulfuretos, com uma barreira de resíduos carbonatados, e fitoestabilização com esteva e/ou rosmaninho dos Tecnossolos elaborados com materiais de *gossan* e resíduos orgânicos/inorgânicos.

PALAVRAS-CHAVE Fitoestabilização • Mina de São Domingos • Plantas autóctones • Resíduos agro-industriais • Tecnossolos

ABSTRACT

The potential rehabilitation of sulfides-rich and *gossan* wastes from São Domingos mine using organic/inorganic residues and plant species with economic value, *Cistus ladanifer* (rockrose) and *Lavandula pedunculata* (lavender) was studied in this work. The ecophysiological behaviour of both species under natural conditions and the quality of some plant-based products (lavender infusion and bioextracts of both species) were compared according to the soils characteristics where the plants grow spontaneously. The efficiency of Technosols, composed of wastes (organic/inorganic and mine materials), under controlled conditions were evaluated in order to improve the chemical quality of the leachates (percolated and simulated) and, consequently, minimize the dispersion of the contaminants, as well as to improve chemical and biological characteristics of the materials for vegetation development.

Independently of soil characteristics, both species grow without signs of toxicity or nutritional deficiency showing appropriate ecophysiological characteristics to their use in phytostabilisation. In both species, the first mechanism of tolerance to potentially hazardous elements from soils is their storage in the roots and low translocation to shoots (sites photosynthetically actives). The phytotoxic concentrations of some elements in the lavender shoots did not affect concentrations of pigments and protein and did not trigger mechanisms of tolerance, enzymatic and non-enzymatic, related to oxidative stress. The products obtained from plants growing in the mining area and an uncontaminated place presented similar quality and, consequently, economic viability for different uses.

Sufide-rich wastes leached extremely acid solutions with higher concentrations of hazardous elements than leachates from gossan wastes. The more sustainable approach to the rehabilitation of both mine wastes from São Domingos was the development of a system that combines the natural isolation of materials rich in sulfides, with a barrier composed of carbonated wastes, and phytostabilisation of Technosols done with gossan materials and organic/inorganic wastes using rockrose and/or lavender.

KEYWORDS Phytostabilisation • São Domingos mining area • Autochthones plants • Agro-industrial residues • Technosols

INTRODUÇÃO GERAL

A actividade mineira em Portugal, nomeadamente na Faixa Piritosa Ibérica (FPI), é antiga porém, a maioria das minas encontram-se, actualmente, abandonadas e constituem graves problemas ambientais e de saúde pública. A mina de São Domingos (SE de Portugal) é uma das minas da FPI que apresenta elevada perigosidade ambiental. Esta, deve-se aos grandes volumes de escombreiras de diferentes tipos, muitas delas instáveis, com granulometria variada, pH ácido e baixa fertilidade, bem como, elevadas concentrações totais de elementos potencialmente tóxicos (EPTs) que podem afectar a saúde humana ou a conservação dos ecossistemas através de diferentes efeitos negativos sobre as águas, solos e biota. Além disso, algumas das escombreiras possuem fases sólidas reactivas, em especial sulfuretos polimetálicos, que após oxidação geram drenagem ácida. Este lixiviado, para além de possuir contaminação multielementar, leva à dissolução de outras fases sólidas promovendo assim a dispersão de elementos potencialmente contaminantes para as áreas adjacentes (solos, sedimentos e águas), os quais consequentemente afectam negativamente a sobrevivência e/ou desenvolvimento da biodiversidade e as funções ecológicas do ecossistema. Assim, o problema ambiental presente na mina de São Domingos e em muitas outras áreas mineiras da FPI relaciona-se não só com as características extremas das escombreiras, mas também com a potencial geração de drenagem ácida.

Embora nestas áreas coexistam diversos factores de stresse para as plantas, associados não só à actividade mineira (elevadas concentrações totais de elementos químicos nos materiais, baixos valores de pH e matéria orgânica), mas também às condições meteorológicas das zonas mediterrâneas (défice hídrico durante a maior parte do ano, elevada radiação solar e temperatura do ar no verão), algumas escombreiras ou solos desenvolvidos sobre estes materiais são colonizadas naturalmente por vegetação espontânea e pioneira. O desenvolvimento desta vegetação tolerante contribui para a génese de solos a partir dos materiais das escombreiras, e para a reabilitação natural das áreas mineiras bem como, para a evolução da sucessão ecológica.

Das várias técnicas de reabilitação ambiental de áreas com contaminação multielementar, a fitoestabilização é considerada a mais eficiente e sustentável. Contudo, o sucesso do estabelecimento de plantas, mesmo que tolerantes e autóctones, directamente nos solos a reabilitar e a longo prazo pode ser um processo difícil em condições de clima árido ou semiárido. Assim, a implementação de um processo conjunto ou de processos independentes que melhorem as condições de reacção do solo, a fertilidade, a retenção de água útil e a estabilização da estrutura do solo, através da adição de diferentes correctivos, pode acelerar a reabilitação através da fitoestabilização.

No entanto, é de salientar que a reabilitação de áreas mineiras abandonadas só será considerada, nos dias de hoje, se incluir não só melhorias ambientais mas também uma abordagem económica. Assim, tendo em conta que distintos resíduos industriais ou agro-industriais não têm qualquer valorização, acabando depositados em aterros ou dispostos clandestinamente no terreno, e ainda a directiva europeia 1999/31/EC relativa à deposição de resíduos orgânicos em aterros, a utilização de resíduos na reabilitação destas áreas é a solução mais sustentável. Além disso, a concepção de um Tecnossolo derivado de diferentes resíduos, elaborado com as características adequadas à solução dos diferentes problemas que limitam ou retardam a reabilitação ambiental, em vez de uso de

resíduos independentes, pode assegurar ou melhorar a recuperação a médio-longo prazo dessas áreas degradadas. É pois a aplicação do conceito de "*Tecnossolo à la carte e à imagem dos solos naturais*" que contribuirá para a recuperação das áreas degradadas o que inclui a reutilização sustentável de resíduos.

Várias espécies de plantas e materiais orgânicos/inorgânicos tem sido sugeridos para a fitoestabilização de solos adjacentes às áreas mineiras ou de solos desenvolvidos sobre escombreiras constituídas por rochas encaixantes e/ou materiais contendo teores baixos de fases sólidas geradoras de acidez. Contudo, não são conhecidas, a nível nacional e internacional, soluções ambientais de recuperação de escombreiras de *gossan* e, em particular, de escombreiras ricas em sulfuretos, estas últimas de granulometria grosseira.

Portanto, devido à complexidade das escombreiras/solos das áreas mineiras, principalmente nas ricas em sulfuretos e em condições mediterrâneas, torna-se essencial que os programas de reabilitação incluam uma análise química multielementar das mesmas, bem como das suas propriedades físico-químicas. É ainda importante o estudo detalhado de espécies vegetais e resíduos (agrícolas, industriais, entre outros) que existam nestas áreas. Esta informação pode ser essencial para usar, gerir e reabilitar estes ambientes que correspondem a focos de contaminação ambiental. A escolha do *Cistus ladanifer* L. (nome comum: esteva) e *Lavandula pedunculata* (Mill.) Cav (nomes comuns: lavandula, lavanda ou rosmaninho) deveu-se ao facto de serem espécies bem adaptadas às condições mediterrâneas, ocorrerem espontaneamente quer em áreas degradadas e contaminadas quer em áreas não-contaminadas e, além disso, possuírem valor económico.

O presente estudo teve como objectivo principal avaliar a potencialidade de reabilitação de dois tipos de escombreiras de áreas mineiras abandonadas, uma rica em sulfuretos e outra de materiais de *gossan*, com recurso a resíduos orgânicos e inorgânicos de baixo valor com vista ao desenvolvimento das espécies *C. ladanifer* e *L. pedunculata* para a exploração de bioextractos vegetais. Deste modo, o trabalho teve como objectivos específicos:

a. Avaliar a capacidade de absorção, acumulação e distribuição dos EPTs nas raízes e parte aérea das plantas estudadas e sua influência nas concentrações de nutrientes, face às concentrações de elementos existentes nos materiais das escombreiras/solos;

b. Comparar algumas respostas fisiológicas de *L. pedunculata* associadas ao stresse oxidativo e alguns componentes envolvidos nos mecanismos de tolerância em plantas crescendo em solos com diferentes níveis de contaminação multielementar;

c. Avaliar a influência dos EPTs na qualidade dos bioextractos vegetais de ambas as plantas estudadas e do chá/infusão de rosmaninho;

d. Inferir sobre a potencial comercialização de produtos extraídos da esteva e rosmaninho usados em programas de fitoestabilização de áreas mineiras da FPI;

e. Avaliar e comparar a eficiência de resíduos agro-industriais, de custo "zero" e facilmente disponíveis numa área relativamente próxima da mina de São Domingos de modo a constituir um Tecnossolo, na melhoria das características físico-químicas e da actividade biológica de escombreiras de *gossan* e de escombreiras ricas em sulfuretos para permitir o melhor desenvolvimento das espécies autóctones e espontâneas estudadas;

f. Avaliar a eficiência dos Tecnossolos estudados (misturas dos materiais de escombreiras com os mesmo resíduos) nas características químicas dos lixiviados e, consequentemente, na minimização da dispersão da contaminação;

g. Estudar o desenvolvimento e comportamento de absorção, acumulação e translocação dos elementos químicos nas plantas crescendo nos Tecnossolos estudados;

h. Avaliar a eficiência de uma reabilitação combinada das duas escombreiras em estudo, através da fitoestabilização com esteva e rosmaninho e concepção de Tecnossolos elaborados com *gossan* e resíduos orgânicos/inorgânicos, e sua aplicabilidade em programas de recuperação ambiental em áreas mineiras da FPI.

Esta tese está dividida em 12 capítulos. No *Capítulo 1* consta a revisão bibliográfica sobre os principais conceitos científicos utilizados neste trabalho e, nos capítulos seguintes (*Capítulos 2−11*) expõem-se os resultados da investigação realizada através de artigos científicos. Os resultados estão agrupados em três temáticas centrais:

i) Estudo do sistema escombreira/solo-planta em condições naturais no qual se compara o comportamento ecofisiológico das plantas de *C. ladanifer* e *L. pedunculata* face às características dos substratos onde estas se desenvolvem espontaneamente (*Capítulos 2−5*);

ii) Estudo do sistema escombreira/solo-planta em condições controladas onde se avalia a eficiência dos Tecnossolos, usando diferentes misturas de resíduos resultantes da agro-indústria e os materiais dos dois tipos de escombreira em estudo (ricas em sulfuretos ou *gossan*), na melhoria da qualidade química dos lixiviados (de percolação e simulados), de modo a minimizar a dispersão dos contaminantes (*Capítulos 6−8*)

iii) Estudo do sistema escombreira/solo-planta em condições controladas onde se compara a eficiência dos Tecnossolos na melhoria das características químicas e biológicas dos materiais de modo a permitir o desenvolvimento de vegetação espontânea (*Capítulo 9−11*).

Assim, no *Capítulo 2* compara-se a variabilidade, ao nível da acumulação e translocação de EPTs e nutrientes (As, Ca, Cu, Fe, K, Mg, Mn, Ni, Pb e Zn), de diferentes populações de *C. ladanifer* crescendo espontaneamente em várias áreas mineiras da FPI (Brancanes, Caveira, Chança, Lousal, Neves Corvo e São Domingos).

No *Capítulo 3* apresenta-se o potencial de *C. ladanifer* na fitoestabilização de solos, desenvolvidos em diferentes substratos, que contêm elementos não essenciais (Al, Ag, Ba, Bi, Cd, Sb e Sr), alguns deles pouco estudados. Para isto é exposto o comportamento de três populações de *C. ladanifer* colhidas nas áreas mineiras de Caveira, Lousal e São Domingos (FPI) ao nível da absorção, acumulação e translocação dos elementos não essenciais e a influência destes nas concentrações dos nutrientes e elementos benéficos (Co, Na e Se). São também tecidas considerações sobre o uso da esteva em programas de fitoestabilização.

No *Capítulo 4* avalia-se o comportamento do sistema solo-planta e as respostas fisiológicas de *L. pedunculata*, crescendo em solos com contaminação multielementar (mina de São Domingos) e em solos não contaminados, associadas ao stresse oxidativo e a vários componentes (enzimáticos e não

enzimáticos) envolvidos em mecanismos ecofisiológicos de tolerância. Isto permitiu avaliar o potencial desta espécie para fitoestabilização.

No *Capítulo 5* comparou-se a qualidade de alguns produtos derivados de *C. ladanifer* e *L. pedunculata* (chá/infusão de rosmaninho e bioextractos vegetais de ambas as espécies) face às concentrações, nestas plantas, de EPTs para a saúde humana.

No *Capítulo 6* avaliam-se as mudanças hidrogeoquímicas e mineralógicas, a médio prazo, face à aplicação de misturas de resíduos orgânicos/inorgânicos a materiais de escombreira ricos em sufuretos. Para isto é apresentada a variação temporal das características dos lixiviados de percolação e dos materiais a diferentes profundidades.

No *Capítulo 7* apresenta-se a variação multielementar de lixiviados simulados de Tecnossolos, preparados com materiais de *gossan* ou ricos em sulfuretos e misturas de resíduos orgânicos/inorgânicos, e o potencial risco ambiental dos mesmos, relativamente aos materiais de escombreira sem reabilitar, tendo em conta a concentração dos EPTs lixiviados e a massa de escombreiras existente na mina de São Domingos.

No *Capítulo 8* avaliou-se a eficiência da sobreposição de uma camada de *gossan* sobre materiais de escombreira rica em sulfuretos e dos Tecnossolos (estudados nos capítulos anteriores), na melhoria da reabilitação dos resíduos mais perigosos ao nível da actividade enzimática da comunidade microbiana dos materiais, qualidade química de lixiviados de percolação e germinação de sementes de *C. ladanifer*.

No *Capítulo 9* expõem-se a influência das taxas de aplicação de uma mistura de resíduos orgânicos/inorgânicos no Tecnossolo, contendo escombreira de *gossan*, na melhoria das características químicas e biológicas dos materiais e no crescimento de *C. ladanifer*.

No *Capítulo 10*, que é baseado nos dados dos capítulos anteriores, apresenta-se uma reabilitação combinada das escombreiras em estudo, através do isolamento natural da escombreira rica em sulfuretos, da fitoestabilização com esteva e rosmaninho e uso de Tecnossolos. É de salientar que estes Tecnossolos foram elaborados com material de *gossan* colhido em outra escombreira, também da área de São Domingos mas que praticamente não apresentava rocha encaixante, e por isso apresentando condições mais extremas, e as misturas de resíduos onde se obteve melhor desenvolvimento das plantas de *C. ladanifer*.

No *Capítulo 11* pretendeu-se estudar o período pós-reabilitação de um solo desenvolvido sob materiais de *gossan* após aplicação de polímeros hidrofílicos de poliacrilato e crescimento de *Spergularia purpurea* (Pearson) G. Don fil. Para isto, avaliaram-se as características químicas e ecotoxicidade do solo bem como a colonização natural por espécies herbáceas e a germinação e desenvolvimento das espécies arbustivas em estudo.

Face aos resultados obtidos em todo o trabalho realizado, no *Capítulo 12* enunciam-se algumas conclusões finais.

1. REVISÃO BIBLIOGRÁFICA

1.1 ACTIVIDADE MINEIRA

Os depósitos minerais, produto de um ou mais ciclos geoquímicos, têm sido, desde tempos imemoriais, uma fonte económica muito importante para o Homem.

Ao longo da história, a actividade mineira, quer desenvolvida a céu aberto ou em lavra subterrânea, originou diferentes tipos de escombreiras resultantes da extracção e processamento do minério e seu encaixante e, ainda, a circulação de lixiviados enriquecidos em elementos potencialmente tóxicos (EPTs) para o meio (Matos e Martins, 2006; Wong, 2003). As escombreiras produzidas são, usualmente, de grande volumetria, instáveis, com granulometria variada, baixo valor de pH e fertilidade e elevadas concentrações totais de metais e metalóides (Abreu e Magalhães, 2009; Freitas et al., 2004a; Matos e Martins, 2006; Wong, 2003). Além disso, as escombreiras com textura grosseira apresentam baixa capacidade de retenção de água incrementando, ainda mais, o forte xerofitismo climático, factor que condiciona a sobrevivência das plantas durante a época seca. Estas características conduzem à modificação das características físicas, químicas e biológicas dos solos adjacentes às áreas mineiras ou dos solos desenvolvidos sobre estes materiais que, consequentemente, afectam a colonização e desenvolvimento da vegetação. Neste sentido, a camada superficial dos solos fica sujeita a um maior risco dos processos erosivos (Abreu e Magalhães, 2009; Freitas et al., 2004a; Tordoff et al., 2000).

De um modo geral, este tipo de actividade e seu abandono podem induzir efeitos directos e indirectos no meio sendo que o clima, temperado a árido/semiárido, contribui para uma potenciação destes efeitos (Mendez e Maier, 2008). Como efeitos directos indicam-se a perda de solo e o decréscimo da biodiversidade específica ou funcional enquanto nos efeitos indirectos se inclui o impacto paisagístico e a degradação e contaminação dos solos, sedimentos, águas e ar que, consequentemente, conduzem à ruptura das funções ecológicas essenciais dos sistemas (Wong, 2003). De facto, a toxicidade dos elementos químicos bem como a diminuição do pH como consequência da natureza dos materiais de escombreiras pode afectar o número, diversidade e a actividade da comunidade microbiana do solo inibindo os processos biogeoquímicos (Hinojosa et al., 2008; Wong, 2003). Dependendo dos elementos e da sua mobilidade, estes podem ser bioacumulados ao longo da cadeia alimentar determinando ainda consequências deletérias nos diferentes níveis tróficos.

A exploração mineira de sulfuretos maciços polimetálicos é uma das que apresenta maiores problemas ambientais. A existência de fases sólidas reactivas, como são os sulfuretos, e a sua contínua oxidação, química e bioquímica, conduz à geração de drenagem ácida de mina (AMD – *acid mine drainage*) a qual está enriquecida em sulfatos bem como EPTs (Abreu et al., 2010a; Álvarez-Valero et al., 2009; Romero et al., 2010; Sanchéz-España, 2008; Sanchéz-España et al., 2005, 2006). Segundo os mesmos autores, a composição química da AMD é extremamente variável dependendo do tipo de depósito e época do ano porém, pode incluir EPTs como o As, Fe, Cu e Zn (Quadro 1).

Em ambiente mediterrâneo e especialmente nos períodos secos de primavera e verão, é usual a formação de eflorescências de sais solúveis à superfície ou nos poros dos materiais (ex. sulfatos de Al, Fe e Mg com diferentes níveis de hidratação, como a epsomite [$MgSO_4 \cdot 7H_2O$], a melanterite

[$FeSO_4$•$7H_2O$], e a copiapite [$Fe^{2+}Fe_4^{3+}(SO_4)_6(OH)_2$•$20H_2O$]) devido à evaporação da AMD nos poros dos materiais (Abreu et al., 2010a; Álvarez-Valero et al., 2008, 2009; Ferreira da Silva et al., 2015; Sanchéz-España, 2008; Sanchéz-España et al., 2005; Valente e Gomes, 2009). Segundo estes autores, estes sais apresentam hidrólise acidificante e funcionam como "*fontes de retenção*" de elementos químicos porém, após as primeiras chuvas, redissolvem-se enriquecendo significativamente a AMD com elementos alterando a sua composição.

A AMD provoca também alterações no ecossistema envolvente devido ao seu pH ser frequentemente baixo (< 3) contribuindo, em geral, para incrementar a dissolução de fases sólidas contendo EPTs e também o seu transporte (Abreu et al., 2010a; Ferreira da Silva et al., 2013; Romero et al., 2010; Sanchéz-España, 2008). A área de dispersão depende de outros factores, como a hidrologia, a geomorfologia e o clima da área, e das características dos elementos químicos podendo atingir distâncias consideráveis em relação à origem (Bleeker et al., 2002). Uma dispersão da contaminação, através das partículas finas de material de escombreira e da AMD, até 2500 m a jusante do local da mina foi referida por Romero et al. (2010). Por outro lado, Sanchéz-España et al. (2005) indicaram que a contaminação associada às áreas mineiras da FPI Espanhola afecta uma área extremamente ampla, entre 104 de 122 km, do curso principal do rio Odiel.

Em suma, este tipo de exploração e o seu abandono tem forte probabilidade de ser uma fonte de contaminação e degradação do ambiente envolvente, caso não sejam tomadas medidas adequadas de gestão ambiental. No entanto, as áreas mineiras podem ser um nicho ecológico para a colonização/evolução de novas espécies, como é o caso da *Erica andevalensis* Cabezudo & Rivera espécie endémica das áreas mineiras da FPI (Abreu et al., 2008; Cabezudo e Rivera, 1980), ou de ecótipos.

Quadro 1 Características químicas da drenagem ácida de algumas minas da Península Ibérica.

Áreas mineiras		g/L			mg/L		
	pH	**SO_4^{2-}**	**Fe**	**Zn**	**As**	**Cu**	**Pb**
FPI (Espanha)[a]	1,3 – 4,2	0,3 – 43,9	4 – 5,8	<LD – 1437	1 – 39,7	0,5 – 440	<0,1 – 0,7
FPI (Portugal)[b]	1,0 – 3,5	9,3 – 20,7	$7,9 \times 10^{-3}$ – 11,5	$1,5 \times 10^{-3}$ – 0,3	<LD − 388	0,1 − 111	<LD – 5,0
La Zarza, Lomero, Esperanza, Corta Atalaya e Poderosa (Espanha)[c]	2,2 – 3,5	2,9 – 33,3	0,3 – 2,1	13 – 568	<LD – 4,7	0,4 –173	0,04 – 0,7
San Telmo (Espanha)[d]	0,6 – 0,8	134,2	74,2	1,1	303	1945	0,1
Tinto Santa Rosa (Espanha)[e]	2,6 – 3,4	2,7 – 4,0	0,2 –1,0	56 – 85	0,03 – 1,9	15 – 23	0,06 – 0,08
Caveira (Portugal)[f]	1,1 – 2,7	0,8 – 49,9	0,1 – 11,1	0,01 – 0,3	$78 – 86,1 \times 10^3$	1,1 – 58,5	$850 – 4,5 \times 10^3$
Panasqueira (Portugal)[g]	2,9 – 3,9	0,7 – 3,7	1,7 – 82,5	12,4 – 49,2	$48 – 2,1 \times 10^3$	$(2,1 – 54,3) \times 10^3$	<22
São Domingos (Portugal)[h]	2,8	1,8	0,07	13×10^{-3}	0,1	28	<0,04
Tinoca (Portugal)[i]	2,5	8,0	0,8	34×10^{-3}	<0,5	138	<0,2
Valdarcas (Portugal)[j]	2,1 – 3,7	0,1 – 21,6	$1,6 \times 10^{-3}$ – 15,0	$0,4 \times 10^{-3}$ – 0,04	6×10^{-4} – 57	0,03 – 45,0	—

[a]Sanchéz-España et al., 2005; [b]Abreu et al., 2010a e demais referências aí indicadas; [c]Sanchéz-España et al., 2006;[d]Sanchéz-España et al., 2008; [e]Asta et al., 2007; [f]Ferreira da Silva et al., 2013; [g]Ávila et al., 2008; [h]Martins et al., 2011; [i]Valente e Gomes, 2009; LD: Limite de detecção

1.2 FAIXA PIRITOSA IBÉRICA: ÁREA MINEIRA DE SÃO DOMINGOS

A Faixa Piritosa Ibérica (FPI) é um dos mais importantes e antigos depósitos de sulfuretos maciços vulcanogénicos do mundo estando localizada a sudoeste da Península Ibérica (Matos e Martins, 2006). Estende-se ao longo de 250 km, desde a Marateca (região do Alentejo − Portugal) até Sevilha (Espanha), sendo caracterizada pela existência de grandes depósitos de sulfuretos polimetálicos que podem atingir mais de 200 Tg, como é o caso de Neves Corvo, Aljustrel e Rio Tinto (Barriga et al., 1997; Matos e Martins, 2003, 2006; Sáez et al., 1999; Tornos, 2006). A actividade mineira nesta área está distribuída por mais de 80 minas, algumas delas com indícios de exploração há mais de 5000 anos (ex. área mineira de Rio Tinto; Ortiz, 2003). Apesar do impacte ambiental de todas as áreas mineiras da FPI ser significativo, as da parte espanhola apresentam uma maior área afectada (Matos e Martins, 2006).

Na parte portuguesa da FPI (FPIP) existem várias áreas mineiras nas quais a extracção foi feita, principalmente, a partir do *gossan* e dos sulfuretos maciços polimetálicos, no entanto actualmente quase na sua totalidade estão abandonadas (Matos e Martins, 2006). Actualmente, Aljustrel e Neves Corvo são as únicas que estão operacionais.

A actividade extractiva nas explorações abandonadas da FPIP decorreu mais intensamente nos séculos XIX e XX, sem quaisquer medidas de gestão ambiental devido à ausência de legislação e consciência ambiental, contudo o impacte no meio é de longo prazo já que houve vários períodos intermitentes de exploração (Abreu et al., 2010a; Matos e Martins, 2006; Quental et al., 2002). O grau de perigosidade ambiental varia consoante a área mineira e tem em conta, principalmente, a tipologia das mineralizações, a volumetria das escombreiras e a sua estabilidade, e as concentrações de EPTs nos materiais das escombreiras bem como o impacte existente nos solos/sedimentos, na rede hidrográfica e na paisagem (Matos e Martins, 2006; Oliveira et al., 2002).

Na mina de São Domingos (SE da FPI, concelho de Mértola), a actividade mineira iniciou-se na época pré-romana com a exploração de Ag, Au e Cu a partir do *gossan*, contudo no século XIX a extracção baseou-se principalmente no Cu e S dos sulfuretos maciços, apesar de também ser explorado Au, Pb e Zn tanto do *gossan* e como dos sulfuretos. Já no século XX e até 1966, data de encerramento da mina, a evolução da indústria do ácido sulfúrico promoveu a extracção do S a partir da pirite e o aumento da exploração de alguns minérios (Quental et al., 2002). A actividade cessou principalmente por questões económicas e sociais, nomeadamente esgotamento do minério (Abreu et al., 2010a; Quental et al., 2002).

Apesar do depósito ser caracterizado de médio porte, a sua longa história de mineração contribuiu para a existência de diferentes resíduos e um impacte ambiental que não se resume ao foco de exploração mineira mas também, a uma vasta faixa que abrange cerca de 50 km^2 (Matos et al., 2006; Quental et al., 2002). Na área mineira de São Domingos, os diversos materiais de escombreira expostos estimam-se em aproximadamente 32 Tg (Fig. 1) e resultaram da extracção (*gossan* e rocha encaixante e dos sulfuretos maciços) e do processamento do minério (aterros industriais, britados de pirite, cinzas de fundição, escórias modernas e romanas, óxidos de ferro e tanques de decantação) e apresentam concentrações totais de EPTs que podem ultrapassar 10 g/kg de As ou de Pb e 2 g/kg de

Sb, atingindo também o Cu, Cd e Zn 2,74 g/kg, 159 mg/kg e 8,9 g/kg, respectivamente (Álvarez-Valero et al., 2008; Pérez-López et al., 2008). Estes mesmos autores, tendo em conta a relação massa/volume e a concentração total ou disponível (extraída com ácido acético) dos EPTs das escombreiras, avaliaram a sua perigosidade categorizando-as segundo uma escala. Assim, tendo em conta que apenas a fracção disponível apresenta um real risco ambiental, Pérez-López et al. (2008) classificou o *gossan* e os britados de pirite como o quarto e quinto, respectivamente, tipos de resíduos mais perigosos na mina de São Domingos.

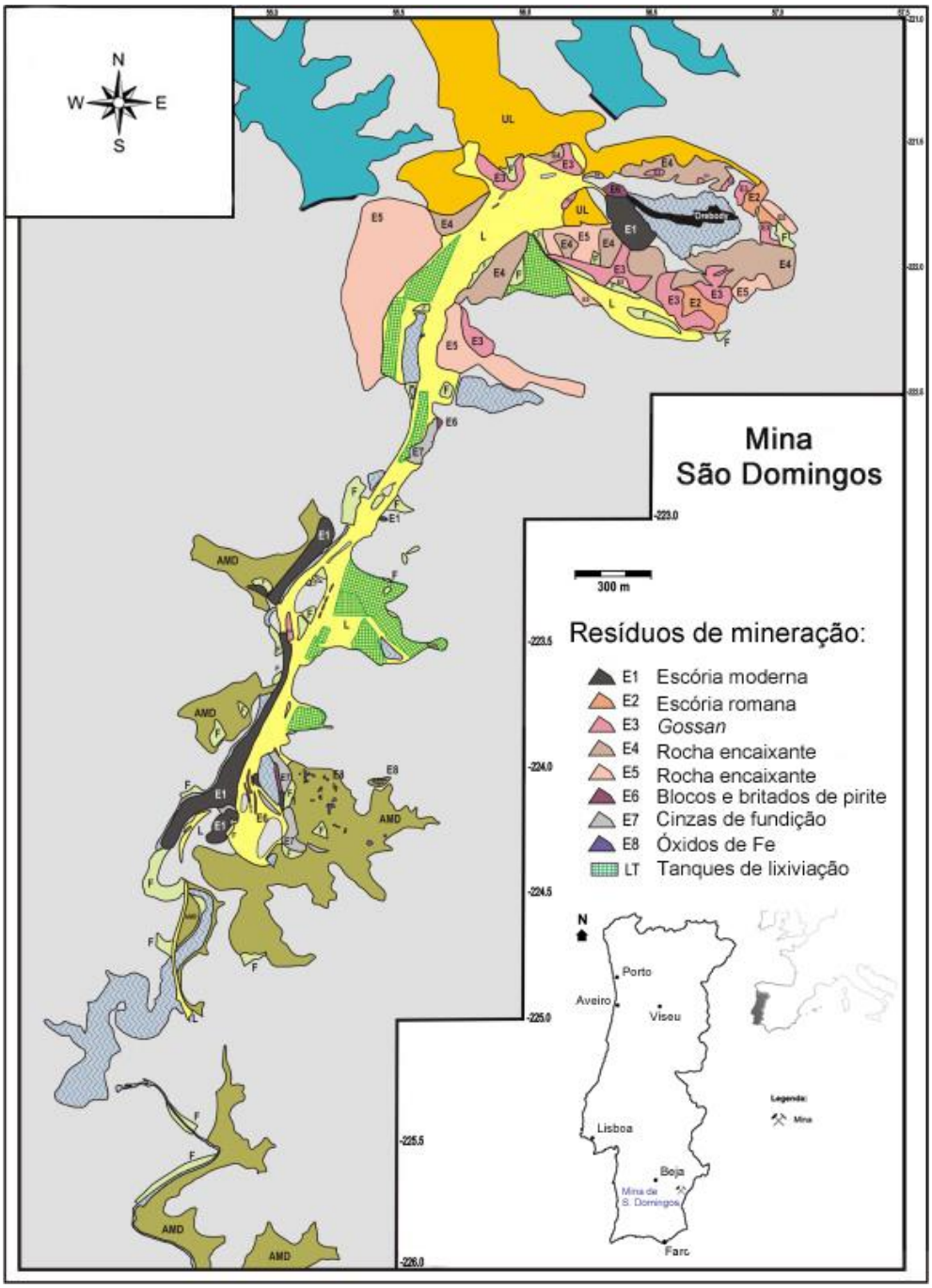

Fig. 1 Mapeamento dos principais tipos de resíduos da mina de São Domingos (Adaptado de Álvarez-Valero et al., 2008).

Os solos das zonas adjacentes e desenvolvidos sobre materiais de escombreira apresentam também contaminação multielementar com elevadas concentrações totais de alguns elementos químicos (Quadro 2), os quais ultrapassam os valores máximos admissíveis para diferentes usos do solo (CCME, 2007), mas com baixa disponibilidade dos mesmos (extraída com diferentes soluções) e pH entre ácido a ligeiramente neutro (Abreu et al., 2008, 2012a,b; Alvarenga et al., 2012, 2014; González-Fernández et al., 2011; Pérez-López et al, 2014; Santos et al., 2012). Além disso, diferentes bioensaios mostraram que alguns solos também possuem um nível de ecotoxicidade relevante (Alvarenga et al., 2012).

No entanto, o problema ambiental presente na área mineira de São Domingos, e em muitas outras áreas mineiras da FPI, relaciona-se não só com as características extremas das escombreiras e dos solos mas também com a potencial geração de drenagem ácida (Abreu et al., 2010a; Álvarez-Valero et al., 2008; Pérez-López et al, 2008; Quental et al., 2002). Esta AMD contém elevadas concentrações de EPTs que rondam 1,48 g Al/L, 4,21 g Fe/L, 2448 g Zn/L e 7,7 g SO_4/L (Abreu et al., 2008). Devido à variabilidade sazonal, a AMD pode atingir concentrações de 416,8 mg Al/L, 24,8 mg As/L, 2,0 mg Cd/L, 182,6 mg Cu/L, 2,95 g Fe/L, 2,2 mg Pb/L, 226 mg Zn/L e 13,9 g SO_4/L (dados não publicados). A lixiviação das várias escombreiras, principalmente nas primeiras chuvas, conduz a um impacte negativo na ribeira de São Domingos, afluente do rio Chança e que drena a área mineira (Matos e Martins, 2006).

Segundo a classificação da perigosidade ambiental em áreas mineiras da FPIP, a mina de São Domingos apresenta o nível máximo (Matos e Martins, 2006) sendo evidente a necessidade de recuperação ambiental da mesma.

Quadro 2 Concentrações totais dos elementos químicos mais representativos em solos da mina de São Domingos (min – max ou média ± DP).

Referências	g/kg		mg/kg			
	As	**Pb**	**Cu**	**Cd**	**Sb**	**Zn**
Abreu et al., 2008	0,01 – 11,6	0,02 – 24,93	15,1 – 989	—	1,5 – 2150	20 – 1369
Abreu et al., 2012a	0,60 – 2,00	1,94 – 7,36	743 – 1750	—	—	342 – 1010
Abreu et al., 2012b	0,50 – 3,03	1,94 – 9,21	210 – 1750	—	69 – 486	33 – 1010
Alvarenga et al., 2012	0,96 – 7,96	1,62 – 26,98	202 – 424	1,9 – 3,4	98 – 364	34 – 168
Alvarenga et al., 2014	0,07 – 1,85	0,27 – 4,95	52,6 – 239,0	—	—	141,4 – 547,8
Freitas et al., 2004a	0,04 – 1,29	0,23 –12,22	87 – 1829	—	—	104 –714
Freitas et al., 2009	0,45 – 3,56	—	—	—	98 – 1099	178 – 4035
González-Fernández et al., 2011	$1{,}1 \times 10^{-3}$ – 7,57	$1{,}2 \times 10^{-3}$ – 18,57	3,2 – 687	—	—	1,9 – 753
Pérez-López et al., 2014	0,08 – 6,89	0,17 – >10,00	1,1 – 62,7	0,2 – 12	5,3 – 1461	33 – 3261
Quental et al., 2002	0,03 – 15,90	0,05 – 32,17	7,64 – 6207	0,15 – 80,01	3,5 – 5640	17,29 – 14850
Santos et al., 2012	2,64 ± 0,61	7,34 ± 1,97	226 ± 14	—	—	43 ± 12
VMA	**0,01**	**0,07 – 0,60**	**63 – 91**	**1,4 – 22**	**20 – 40**	**200 – 360**

[a]VMA: valor máximo admissível para diferentes usos do solo (agricultura, residencial, comercial/industrial) segundo directrizes da qualidade do solo canadense para a protecção do ambiente e da saúde humana (CCME, 2007)

1.3 DISPONIBILIDADE DOS ELEMENTOS QUÍMICOS E SUA TOXICIDADE

Os elementos químicos existentes no solo podem ter origem natural, devido à meteorização da rocha onde estão presentes, ou estar associados às actividades antrópicas, nomeadamente a actividade mineira. Os solos desenvolvidos sobre materiais de escombreiras (Tecnossolos; IUSS Working Group WRB, 2007), ou contaminados por deposição de lamas, material particulado ou escórias de pirite ou de outros resíduos industriais, são também afectados pelas características desses materiais. Porém, a composição química dos solos naturais está dependente das características da rocha original e das condições climáticas (Kabata-Pendias, 2011), entre outros parâmetros.

O conteúdo total de elementos químicos no solo/escombreira é, em regra, apenas um indicador da sua contaminação pois, a concentração dos elementos existentes no meio pode não corresponder e, mesmo, não estar relacionada com a fracção realmente disponível para os organismos (Adriano, 2001; Kabata-Pendias, 2011). Contudo, este valor é usado em diversos países como parâmetro, na maioria da legislação, para a protecção e qualidade do solo.

Os elementos químicos no solo estão associados a distintos componentes que se encontram distribuídos pelas diferentes fases do solo (ex. carbonatos, matéria orgânica, óxidos de ferro e manganês, minerais com solubilidade muito baixa - fase residual) ligando-se através de vários mecanismos, muitas vezes complexos e variados, tais como adsorção, troca iónica, co-precipitação e complexação (Brümmer, 1986). Apenas os elementos associados à fase líquida, constituindo a fracção solúvel, e ao complexo de troca de colóides inorgânicos e orgânicos estão disponíveis para os organismos, variando no espaço e no tempo (Adriano et al., 2004; Kabata-Pendias, 2004).

Análises de fraccionamento, via sequencial (Berti e Jacobs, 1996; Rauret et al., 1999; Tessier et al., 1979) ou por extracção parciais (Adriano et al., 2004), são muitas vezes realizadas para avaliar a redistribuição dos elementos pelas distintas fases do solo (Abreu et al., 2009, 2010b, 2012a; Lázaro et al., 2006; Pérez-López et al., 2008; Santos et al., 2012) e, consequentemente, avaliar a disponibilidade real ou potencial dos mesmos (Kabata-Pendias, 2004). Para a determinação da fracção disponível para as plantas, diferentes metodologias têm sido definidas (Feng et al., 2005; Kabata-Pendias 2004, 2011; Lindsay e Norvell, 1978; Meers et al., 2007; Schollenberger e Simon, 1945) porém, estas extracções baseiam-se, principalmente, em testes de solo (McLaughlin et al., 2000) não dando uma indicação sobre o sistema solo-planta na zona da rizosfera (Kabata-Pendias, 2011). De acordo com alguns autores (Feng et al., 2005; Wang et al., 2004), as espécies vegetais são um factor importante a ter em conta. Além disso, a fracção disponível varia com a solução aquosa extractante, elemento e as características do solo não mostrando usualmente um padrão regular (Adriano et al., 2004; Anjos et al., 2012; Lázaro et al., 2006; Meers et al., 2007; Kabata-Pendias 2004; Santos et al., 2012).

Vários factores podem regular a disponibilidade dos elementos, nomeadamente físicos (ex. constituição da fracção coloidal), químicos (ex. pH e condições de oxi-redução), biológicos (ex. actividade microbiana na área da rizosfera ou exsudados das raízes) e a interacção entre os mesmos (Bolan et al., 2014; Ernst, 1996; Kabata-Pendias, 2004; Hinsinger et al., 2006) (Fig. 2).

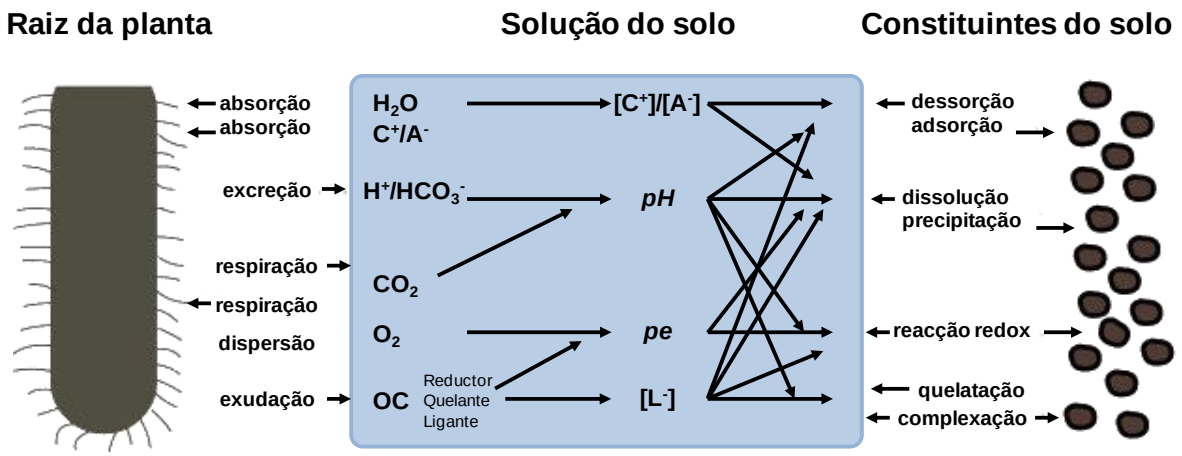

Fig. 2 Esquema de possíveis factores que influenciam a disponibilidade dos elementos químicos nos solos (Adaptado de Adriano et al., 2004).

Os baixos valores de pH dos lixiviados ou dos materiais das áreas mineiras, especialmente as de sulfuretos, podem promover um aumento da disponibilidade dos elementos no meio (Dinelli e Lombini, 1996; Hinojosa et al., 2008).

Para certa gama de pH, a disponibilidade também é dependente de outros parâmetros nomeadamente da presença de oxi-hidróxidos de ferro e manganês. Estes compostos têm a capacidade de adsorver os elementos, através da formação de complexos de esfera interna, ou oxidar directamente ou catalisar a oxidação de outros elementos (ex. Cr e As) conduzindo, deste modo, a uma diminuição da disponibilidade dos elementos (Bolan et al., 2004; Dinelli e Lombini, 1996; McBride, 1989). O possível papel dos oxi-hidróxidos de ferro na retenção de aniões, por exemplo o As, é indicado por vários autores (Bolan et al., 2014; Kabata-Pendias, 2011).

O conteúdo de matéria orgânica pode também reduzir a disponibilidade dos elementos devido à presença de grupos quelatantes, aumento da capacidade de troca catiónica ou mudança do estado de oxidação de alguns elementos (Adriano, 2001; Kabata-Pendias, 2011; Tejada et al., 2008). De facto, a aplicação de diferentes resíduos orgânicos (ex. composto de resíduos sólidos urbanos − RSU, biossólido ou lamas de estação de tratamento de águas residuais − ETAR) em solos contaminados pode diminuir a concentração de elementos na fracção disponível, usualmente catiões (Pérez-de-Mora et al., 2005). Contudo, é de salientar que a extensão da formação dos complexos organo-metálicos depende de factores como a temperatura, tipo de matéria orgânica e concentração dos elementos na solução do solo (Bolan et al., 2014). Neste contexto, Tejada et al. (2008) verificou que, apesar da adição de alguns resíduos orgânicos (composto de restos da cultura do algodão - *crushed cotton gin*, e estrume de galinha) a um solo contaminado artificialmente com doses crescentes de Ni diminuir o efeito inibitório deste elemento na actividade enzimática e na biomassa microbiana, o ED50 (*ecological dose*: concentração do elemento para a qual a actividade biológica é reduzido 50 %) era maior no tratamento com o composto. Este facto parece relacionar-se ao maior conteúdo de ácidos húmicos neste resíduo e, consequentemente, maior capacidade de adsorção de Ni (comparativamente ao outro resíduo que apresentava maior conteúdo de ácidos fúlvicos) (Tejada et al., 2008). É ainda de salientar que o aumento do conteúdo de carbono orgânico dissolvido (DOC)

pode aumentar a disponibilidade do As e, durante o processo de mineralização da matéria orgânica, os elementos podem ser libertados novamente para o meio (Bolan et al., 2014).

A presença de plantas e de toda a comunidade microbiana associada à rizosfera também pode afectar a disponibilidade dos elementos no meio. No entanto, esta influência mútua é complexa e pouco conhecida (Barceló e Poschenrieder, 2003). As plantas absorvem os iões do solo mas também podem acidificar a solução do solo na zona da rizosfera, através da exsudação de iões H^+ e da respiração das células deste órgão, o que, consequentemente, aumenta a disponibilidade dos elementos (Ali et al., 2013; Hinsinger et al., 2006). Por outro lado, também são exsudados diferentes compostos orgânicos de baixa massa molecular que aumentam ou diminuem a disponibilidade dos elementos devido a contribuírem para a dissolução de algumas fases sólidas ou funcionarem como complexantes (Bienfait et al., 1982; Hinsinger et al., 2006). De um modo geral, todas estas variações ao nível da rizosfera permitem disponibilizar os nutrientes aquando de desequilíbrio nutricional da planta ou reduzir a disponibilidade de EPTs.

Alterações na qualidade dos solos/escombreiras podem ser medidos através de diferentes indicadores associados aos processos e características físicas, químicas e biológicas dos mesmos (Araújo et al., 2007; Martínez-Salgado et al., 2010). A determinação dos elementos na fracção disponível do meio (solúvel + complexo de troca), através de uma extracção química, continua a ser o indicador chave na avaliação do potencial risco ambiental e de saúde humana ou da qualidade do substrato, especialmente para locais contaminados, mas esta pode variar como indicado anteriormente. Além disso, esta análise não é suficiente para avaliar os efeitos ecotoxicológicos nos organismos e na sua actividade biológica pois, não considera as interacções entre contaminantes, o efeito da matriz e o biota. Logo, qualquer estratégia para avaliação ambiental também deve considerar a realização de bioensaios (Abreu et al., 2014a; Alvarenga et al., 2008a,b, 2012; Adriano et al., 2004; García-Lorenzo et al, 2009; Rocha et al., 2011) que, preferencialmente, incluam organismos de diferentes níveis tróficos e compartimentos terrestres e aquáticos, e/ou indicadores de funcionamento do solo (Hinojosa et al., 2008).

Também as enzimas do solo catalisam diferentes reacções bioquímicas envolvidas nos processos de mineralização e de ciclagem dos nutrientes, podendo a sua actividade ser afectada mais rapidamente pela contaminação ou mudanças de gestão do solo que outras características químicas dos materiais (Araújo et al., 2007; Bandick e Dick, 1999; Belyaeva et al., 2005; de Varennes et al., 2009; Martinez-Salgado et al., 2010). Vários trabalhos mostram os rápidos efeitos deletérios dos elementos químicos sobre as actividades enzimáticas do solo após contaminação artificial de solos com soluções padrão (Belyaeva et al., 2005; Tejada et al., 2008) ou por resíduos de mina (Hinojosa et al., 2008; Pérez-de-Mora et al., 2005). Por outro lado, apesar das variações sazonais normais da comunidade microbiana do solo, vários autores (Hinojosa et al., 2008; Pérez-de-Mora et al., 2005; de Varennes et al., 2010) avaliam a reabilitação de solos contaminados após aplicação de correctivos orgânicos através da actividade enzimática.

1.4 CONCENTRAÇÃO DOS ELEMENTOS QUÍMICOS NAS PLANTAS E RESPOSTAS ECOFISIOLÓGICAS

A composição química das plantas é a resposta a um conjunto de parâmetros, designadamente a disponibilidade dos elementos no solo (associados à fracção solúvel e ao complexo de troca) ou a deposição atmosférica, e pela capacidade de absorver e transferir os mesmos elementos para as diferentes partes da planta através dos seus vasos condutores (Kabata-Pendias, 2011). Assim, a composição química das plantas nem sempre reflecte as concentrações dos elementos no meio, nomeadamente concentrações totais ou disponíveis dos elementos no solo. De facto, a absorção, translocação e acumulação dos elementos nas plantas pode variar com a espécie, ecótipo, população, idade da planta (ou fase de crescimento) e elemento em questão (Abreu et al., 2012a,b; Conesa et al., 2006; Freitas et al., 2004a,b, 2009; González-Fernández et al., 2011; Kabata-Pendias, 2011; Kidd et al., 2004; Lázaro et al., 2006; Santos et al., 2012). É de salientar que algumas condições meterológicas (ou sazonais) também afectam estes mecanismos, tais como o regime hídrico e a temperatura do ar, e desequilibrios nutricionais (Kabata-Pendias, 2011), nomeadamente as que ocorrem em áreas áridas e semiáridas. Por outro lado, as plantas desenvolveram durante a sua evolução vários mecanismos que resultaram na adaptação e tolerância a novos meios ou a meios quimicamente desequilibrados (ex. nutricionalmente ou pela contaminação) (Kabata Pendias, 2004, 2011).

Do ponto de vista biológico, os elementos podem categorizar-se em dois grandes grupos segundo as funções que exercem no metabolismo: os nutrientes (ou elementos essenciais) e os elementos não essenciais (ou potencialmente tóxicos) (Adriano, 2001). Apesar dos mecanismos de absorção pelas raízes poderem ser regulados pela selecção biológica dos elementos, estes processos podem ser limitados perante o elemento (Kabata-Pendias, 2011). Assim, a raiz pode actuar como barreira impedindo a passagem de alguns elementos, contudo outros elementos não-essenciais, devido a possuírem o mesmo estado de valência ou raio iónico semelhante, podem competir efectivamente e entrar nas raízes pelos mesmos transportadores transmembranares (Ghosh e Singh, 2005; Hodson, 2012; Kabata-Pendias, 2011). De facto, é conhecido que, por exemplo, o As(V) funciona como um análogo do P sendo absorvido pelas plantas através do sistema transportador do P (Bolan et al., 2014; Hodson, 2012).

Após a absorção, os nutrientes são usualmente translocados para a parte aérea, onde são incluídos nas diferentes vias metabólicas, enquanto que os elementos não essenciais são retidos nas raízes contudo, em alguns casos pode-se observar o contrário. Vários trabalhos mostram esta variabilidade intra e inter-específica nos comportamentos de absorção e translocação face à importância metabólica dos elementos (Abreu et al., 2012b; Alvarenga et al., 2004; de la Fuente et al., 2010; Kidd et al., 2004; Trigueros et al., 2012).

Ao nível da acumulação nos tecidos fotossinteticamente activos os elementos essenciais, nomeadamente os micronutrientes, podem também ser tóxicos quando a sua concentração ultrapassa a gama de tolerância. Este intervalo de concentração difere com a espécie, elemento ou

mesmo estado de oxidação (Kabata-Pendias, 2011), sendo a gama de tolerância considerada como a gama de concentração para a qual o crescimento e desenvolvimento de determinada espécie são máximos.

Em condições de stresse, como é toxicidade por metais/metalóides, vários processos podem ser afectados devido a um aumento da produção de espécies reactivas de oxigénio (ROS – *reactive oxigen species*) e sua acumulação nas células. A amplitude da resposta depende da intensidade e tempo de exposição ao elemento, formas químicas existentes no solo e espécie da planta (Bourrelier et al., 1998).

A redução do crescimento (ex. comprimento e biomassa da parte aérea) e o aparecimento de cloroses ou necroses são alguns dos efeitos visuais de toxicidade referenciados (Adriano, 2001; Cao et al., 2004; Kabata-Pendias, 2011; Nadgórska-Socha et al., 2013). No entanto, em algumas espécies (ex. *Erica andevalensis*; *Cistus ladanifer*; *Nerium oleander* L.) podem não ser evidentes os sinais visuais de toxicidade, apesar de estarem a ser afectados negativamente alguns processos fisiológicos (ex. alteração do teor de água, diminuição dos conteúdos em clorofilas, desequilíbrio da composição elementar) pelos ROS produzidos pelo excesso de elementos acumulados nos tecidos (Kidd et al., 2004; Mingorance et al., 2012; Santos et al., 2013; Trigueros et al., 2012). Os ROS são compostos altamente oxidantes que provocam danos significativos ao nível fisiológico e celular (ex. processos de transporte, fotossíntese, estrutura das membranas, peroxidação lipídica ou desnaturação de proteínas) (Zitka et al., 2013).

Quando uma planta está sob stresse, face a concentrações de elementos acima da sua gama de óptimo, podem desencadear-se uma série de mecanismos de tolerância (Abreu et al., 2014). Além disso, os elementos químicos acumulados na planta podem conduzir a uma série de efeitos de antagonismo e sinergismo (Kabata-Pendias, 2011). A tolerância pode desencadear-se por estratégias de exclusão, desintoxicação ou tolerância bioquímica (Baker, 1981; Baker e Walker, 1990; Barceló e Poschenrieder, 2003; Levitt, 1980). Segundo estes autores, a estratégia de exclusão é caracterizada como a restrição da absorção dos elementos através da exsudação de compostos orgânicos, limitação da sua translocação para a parte aérea e locais fotossinteticamente activos, e/ou acumulação dos elementos nas folhas mais velhas que senescem e depois caem. Ao nível da raiz, os elementos químicos podem ser acumulados no interior ou sobre a superfície das raízes sendo o plasmalema a primeira barreira para a selecção e o controlo da absorção (Adriano, 2001). Sharma and Dubey (2005) referiram que, em alguns casos, a retenção de Pb nas raízes é baseado na sua precipitação extracelular sob a forma de fosfatos e carbonatos de Pb, acumulados nas paredes celulares, e devido à sua ligação em sítios permutáveis sobre as células. Vários estudos realizados com plantas crescendo em áreas mineiras ou em hidroponia com concentrações crescentes de EPTs mostraram uma limitação da translocação dos elementos devido à retenção dos mesmos principalmente na raiz (Kidd et al., 2004; Pérez-López et al., 2014; Rossini Oliva et al., 2009; Santibáñez et al., 2008; Santos et al., 2009; Trigueros et al., 2012). Por outro lado, a acumulação progressiva de As, Pb e Zn com o desenvolvimento das folhas de *C. ladanifer* ou de As e W em folhas de *Pinus pinaster* Aiton, crescendo em áreas mineiras, leva a que as folhas senescentes tenham as maiores concentrações (Favas et al., 2014; Santos et al., 2013).

A quelatação dos elementos na parede celular ou no citoplasma e compartimentação dos mesmos no vacúolo, principalmente da parte aérea, funcionam como mecanismos de desintoxicação e de redução da disponibilidade dos mesmos nos processos metabólicos essenciais (Barceló e Poschenrieder, 2003). A localização de Pb e Fe maioritariamente na raiz de *E. andevalensis* confirma que a imobilização ao nível deste órgão, nomeadamente em alguns tecidos (Pb: tecidos da epiderme da raiz; Fe: parede celular da epiderme da raiz), funciona como principal mecanismo de tolerância para que as vias metabólicas sensíveis da parte aérea não sejam afectadas (Mingorance et al., 2012; Rossini Oliva et al., 2009). Ainda para a mesma espécie, Trigueros et al. (2012) observou uma maior concentração de Cu e Pb na casca da raiz que na parte interior (cortex e sistema vascular) do mesmo órgão.

Por último, a tolerância bioquímica é assinalada pela presença de vias metabólicas especializadas relacionadas com a síntese ou estimulação de componentes enzimáticos e não-enzimáticos que removem e/ou neutralizar os ROS (Abreu et al., 2014b). A estimulação da actividade de diferentes enzimas (catálase − CAT, superóxido dismutase – SOD, peroxidase – POD) como resposta ao stresse oxidativo provocado pela acumulação de concentrações elevadas de EPTs, em plantas crescendo naturalmente em áreas mineiras ou em ensaios com solos contaminados, é referenciado por vários autores (Cao et al., 2004; Márquez-García e Córdoba, 2009; Nadgórska-Socha et al., 2013; Pang et al., 2003; Santos et al., 2009; Słomka et al., 2008).

Em geral, a actividade de enzimas antioxidativas aumenta até conteúdos de elementos toleráveis e, quando as concentrações excedem este nível uma diminuição subsequente da actividade das enzimas ocorre (Cao et al., 2004). Isto acontece devio à existência de um desequilíbrio entre a geração de ROS e a sua desintoxicação. A existência de uma interligação funcional entre diferentes enzimas também é referenciada, sendo que, por exemplo, a SOD é o maior eliminador de ROS cuja transformação gera H_2O_2 enquanto a CAT e a POD estão envolvidas na remoção de H_2O_2 gerado pela SOD (Abreu et al., 2014b; Cao et al., 2004; Grant e Loake, 2000). Algumas enzimas podem ainda estar presentes em diferentes localizações dentro da célula da planta (ex. em solução nos meios aquosos da célula ou ligada a componentes da parede celular através de interacções electrostáticas) sugerindo diferentes funções fisiológicas, como observado para o *C. ladanifer* (Santos et al., 2009).

Vários componentes não enzimáticos, tais como tióis totais, prolina, glutationas, entre outros, são indicados como eficazes inibidores de ROS ou como constituintes capazes de se ligar a iões metálicos formando complexos menos tóxicos (Abreu et al., 2014b; Ali et la., 2013; Teklić et al., 2008; Zitka et al., 2013). De facto, o aumento dos teores de tióis totais, glutationase e prolina é indicado como mecanismos de tolerância face ao aumento de Ni e Zn em folhas e caules de *Vicia faba* L. cultivadas em solo contaminado artificialmente (Nadgórska-Socha et al., 2013). Por outro lado, Cao et al. (2004) registaram tendência semelhante entre o conteúdo de antioxidantes não enzimáticos (glutationas e tióis) e o aumento das concentrações de As no solo e, consequentemente, maior conteúdo do mesmo elemento na raiz e folhas de *Pteris vittata* L..

1.5 TECNOLOGIAS DE REABILITAÇÃO DE SOLOS/ESCOMBREIRAS CONTAMINADOS COM ELEMENTOS QUÍMICOS

Uma vez que os elementos químicos não se degradam, estes acumulam-se no meio e, consequentemente, provocam efeitos deletérios nos organismos e nas funções dos ecossistemas. Além disso, muitos são os efeitos nocivos dos elementos químicos na saúde humana (Ali et al., 2013). Assim, é evidente a necessidade de remediação/reabilitação dos meios degradados/contaminados.

Para reabilitação de solos contaminados por metais e metalóides existem várias tecnologias (Ali et al., 2013; Bech et al., 2014; Khan et al., 2004). Para a sua selecção é fundamental possuir o máximo de informação sobre o problema (ex. distribuição da contaminação) bem como, a caracterização do local (ex. geológica, climática e geomorfológica) e dos materiais (ex. composição química) (Bech et al., 2014). O custo e a eficiência da técnica são factores que, evidentemente, têm que ser tidos em conta.

As tecnologias convencionais são baseadas em processos físico-químicos associados à engenharia (ex. encapsulação, vitrificação, tratamento electrocinético, entre outras) apresentando grandes limitações, nomeadamente elevado custo de implementação, mudanças irreversíveis nas características do solo (ex. estrutura, fertilidade, comunidade microbiana/microflora) e necessidade de restauração do local, sendo apropriada apenas em pequenas áreas (Abreu e Magalhães, 2009; Adriano et al., 2004; Barceló e Poschenrieder, 2003; Khan et al., 2004). Além disso, algumas técnicas também podem conduzir a uma contaminação secundária (Ali et al., 2013; Ghosh e Singh, 2005). Estas limitações ainda se acentuam mais aquando da reabilitação de áreas com contaminação multielementar e com materiais geradores de drenagem ácida (ex. solo/escombreiras das áreas mineiras da FPI) sendo a eficiência muito baixa a médio prazo.

Segundo Sáinz et al. (2003), a geração de drenagem ácida na FPI é um problema ambiental grave sendo o seu tratamento insustentável devido à sua extrema acidez, concentrações multielementares elevadas bem como à existência de uma vasta área de escombreiras geradoras de AMD. De facto, nas últimas décadas a Junta da Andaluzia fez um considerável investimento na remediação e reabilitação de algumas escombreiras com vista à redução do impacto ambiental da AMD, porém estas tentativas foram ineficazes (Sanchéz-España et al., 2005).

Das outras técnicas existentes, designadas em alguns casos como tecnologias verdes ou *eco-friendly* (globalmente designadas por fitorremediação ou fitotecnologias), são usadas plantas no processo de reabilitação, o qual tem um menor custo de implementação comparativamente às técnicas indicadas anteriormente (Ali et al., 2013; Barceló e Poschenrieder, 2003; Mendez e Maier, 2008). De um modo geral, as técnicas de fitorremediação (Fig. 3) baseiam-se no uso de plantas *in situ* (o que envolve inevitavelmente também a comunidade de microorganismos da rizosfera) para reduzir e/ou estabilizar os elementos existentes no meio (solos, águas ou sedimentos) e, consequentemente, o seu efeito tóxico para ambiente (Barceló e Poschenrieder, 2003; Favas et al., 2014).

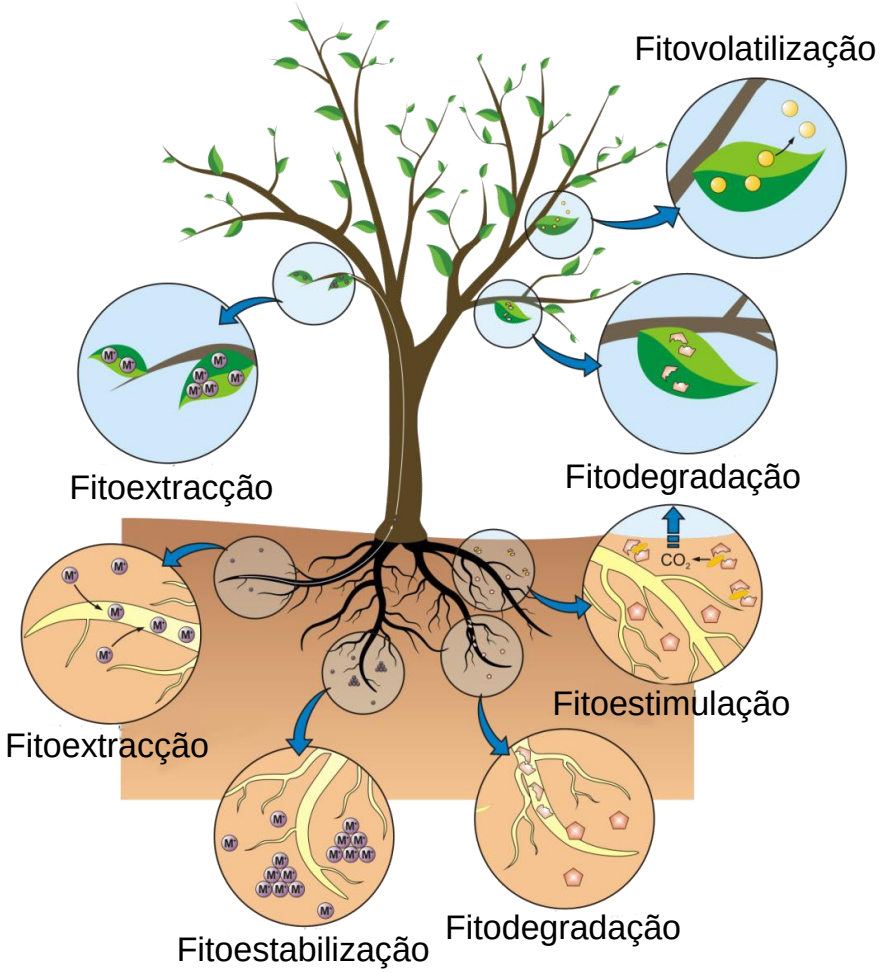

Fig. 3 Esquema das técnicas de fitorremediação existentes (Adaptado de Favas et al., 2014).

De entre as fitotecnologias, a fitoestabilização é a que maior eficiência apresenta na reabilitação de áreas mineiras com contaminação multielementar em climas temperados, áridos e semiáridos (Abreu e Magalhães, 2009; Mendez e Maier, 2008; Tordoff et al., 2000). Assim, nesta técnica utilizam-se plantas tolerantes, com ou sem adição de correctivos (materiais inorgânicos e/ou orgânicos) ao solo/escombreira, com o objectivo de reduzir a disponibilidade dos EPTs através da imobilização dos mesmos por processos de absorção/acumulação pelas raízes bem como precipitação, complexação ou redução na rizosfera (Ali et al., 2013; Abreu e Magalhães, 2009; Adriano et al., 2004; Barceló e Poschenrieder, 2003; Favas et al., 2014; Ghosh e Singh, 2005; Mendez e Maier, 2008; Tordoff et al., 2000; Wong 2003) (Fig. 4).

Deste modo, apesar da concentração total dos elementos no solo permanecer inalterada o potencial risco de exposição é reduzido significativamente (Ghosh e Singh, 2005). Ainda como vantagens, e segundo os autores anteriormente citados, refere-se que o desenvolvimento de plantas contribui para minimização da erosão superficial, do impacto visual na paisagem e da lixiviação bem como da melhoria das características dos substratos através do aumento do conteúdo em matéria orgânica e capacidade de retenção de água, recuperação/promoção da estrutura do solo e estimulação do desenvolvimento de comunidades de microfauna/microbiana relevantes nos processos biológicos. De facto, de Varennes et al. (2010) observou um aumento rápido (ensaio de

microcosmo de seis meses) da actividade de algumas enzimas (ex. desidrogenase, celulase, fosfatase, β-glucosidase e urease) em solos, desenvolvidos sobre *gossan* da mina de São Domingos, apenas com o crescimento de *Dactylis glomerata* L. cv. Amba. Por outro lado, o uso específico de espécies pioneiras pode permitir a modificação do meio degradado facilitando a colonização de outras espécies de plantas com maiores exigências.

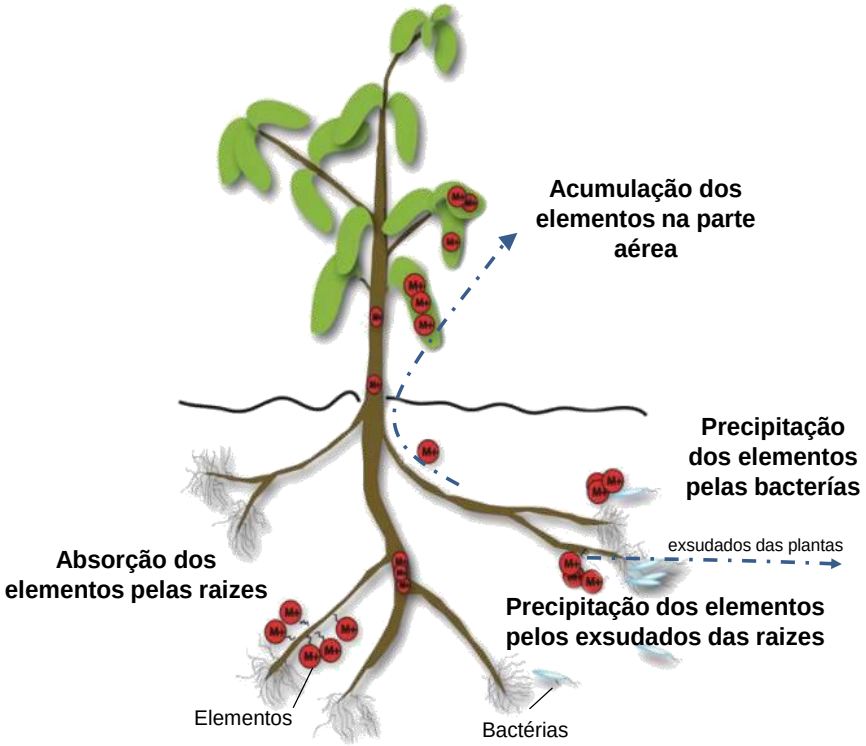

Fig. 4 Esquema dos processos que ocorrem na fitoestabilização (Adaptado de Mendez e Maier, 2008).

A capacidade de tolerância das espécies, não só às concentrações dos EPTs no solo mas também às condições climáticas, é uma das características mais importantes que determina o desenvolvimento e a densidade da vegetação no processo de fitoestabilização. A associação de observações geobotânicas bem como, da avaliação da morfologia das plantas e seu comportamento ecofisiológico é de extrema importância para a selecção de espécies que podem ser utilizadas em programas de fitoestabilização. De um modo geral, as plantas a usar nesta técnica devem ter uma estratégia de excluidoras (*excluders*), e não indicadoras ou hiperacumuladoras, a qual se caracteriza pela acumulação dos elementos na raiz restringindo a sua translocação para a parte aérea (coeficiente de translocação < 1), o que mantem a concentração baixa nos órgãos da parte aérea independentemente da gama de concentração dos mesmos elementos no solo (Ghosh e Singh, 2005). Além disso, segundo Mendez e Maier (2008), os critérios chave para selecção de espécies para fitoestabilização são: concentração dos elementos na parte aérea em valores inferiores ao limite de toxicidade para os animais domésticos (indicados por NRC, 2005) e serem plantas não acumuladoras de elementos (coeficiente de acumulação < 1), com boa cobertura de solo, perenes e com um sistema radicular denso.

Vários estudos têm sido realizados com o objectivo de avaliar o comportamento de plantas autóctones e espontâneas com potencial para a fitoestabilização, nomeadamente para áreas mineiras

com clima mediterrâneo (Abreu et al., 2008, 21012a,b; Anawar et al., 2011; Conesa et al., 2006; de la Fuente et al., 2010; Favas et al., 2014; Freitas et al. 2004a; 2009; Kidd et al., 2004; Lázaro et al., 2006; Madejón et al., 2009; Pérez-López et al, 2014; Rossini Oliva et al., 2009; Trigueros et al., 2012). O uso de comunidades vegetais autóctones e espontâneas de ambientes mineiros mediterrâneos pode assegurar a complementaridade de funções no ecossistema. Além disso, se estas plantas possuírem interesse económico é uma mais-valia no processo, pois estão adaptadas às condições de stresse podendo beneficiar a economia local e amortecer os custos iniciais de implementação. Plantas de crescimento rápido e com grande acumulação de biomassa e de fibras não-alimentares são, por vezes, sugeridas para a reabilitação de áreas contaminadas (Tang et al., 2012).

Vários correctivos (resíduos ou compostos químicos) têm sido testados e/ou usados para a reabilitação, principalmente, de solos (Quadro 3). Contudo, apesar de muitos destes meios apresentarem contaminação multielementar, os estudos existentes apenas se focam em alguns elementos, usualmente catiões.

O princípio básico da aplicação de correctivos é estimular/promover alguns processos de imobilização dos elementos, os quais acontecem naturalmente nos solos (ex. adsorção, complexação e/ou precipitação), reduzindo não só a sua disponibilidade para os organismos mas também a sua potencial lixiviação (Adriano et al., 2004; Kumpiene et al., 2008). Geralmente, a aplicação de correctivos (ex. composto ou resíduos orgânicos/inorgânicos) em solos e/ou escombreiras contaminadas tem um efeito positivo sobre as suas propriedades físicas (ex. melhoria da estrutura), químicas (ex. aumento do valor de pH, matéria orgânica e nutrientes, diminuição da concentração disponível dos EPTs) e/ou biológicas (ex. aumento das actividades enzimáticas e biomassa microbiana) (Abreu e Magalhães, 2009; Adriano et al., 2004; Alvarenga et al., 2008a; Burgos et al., 2010; de Varennes et al., 2010; Hinojosa et al., 2008; Madejón et al., 2009; Pérez-de-Mora et al., 2005; Tejada et al., 2008; Tordoff et al., 2000; Wong, 2003;). Igualmente importante é a contribuição dos correctivos no aumento da biomassa acumulada das plantas (de Varennes et al., 2009, 2010; Alvarenga et al., 2008a; Pérez-de-Mora et al., 2011; Santibáñez et al., 2008).

A diminuição da disponibilidade dos elementos, especialmente em substratos contendo alta concentração de catiões (ex. Cu, Pb ou Zn), através da precipitação dos mesmos pela aplicação de resíduos/compostos carbonatados (ex. cinzas de biomassa, *sugarbeet lime* ou cal) é um dos processos correctivos mais comuns (Adriano et al., 2004; Bolan et al., 2014; Burgos et al., 2010; Clemente et al., 2006; Kumpiene et al., 2008; Mendez e Maier, 2008; Pérez-López et al., 2007; Rodríguez-Jordá et al., 2010; Tordoff et al., 2000; Wong, 2003), contudo esta pode ser uma solução temporária caso os materiais sejam geradores de acidez. O efeito resultante da aplicação destes materiais sobre a imobilização dos elementos químicos relaciona-se com as mudanças relativas de pH e, consequentemente, precipitação dos elementos como hidróxidos ou carbonatos. Além disso, é de salientar que estes compostos podem ter apenas um efeito ténue na reabilitação dos solos. Neste sentido, Rodríguez-Jordá et al. (2010) verificaram que a aplicação de resíduos industriais ricos em carbonatos (Quadro 3) apenas reduzia a lixiviação do Ni, Pb e Zn e não propriamente a fracção do complexo de troca (fracção disponível extraída com DTPA).

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Quadro 3 Alguns exemplos de correctivos usados/testados na reabilitação de solos contaminados.

Materiais a reabilitar	Correctivos	Elementos	Ensaio e espécies usadas
Solos contaminados da mina de Aljustrel[a]	Lamas de ETAR Resíduo da fabricação de açúcar de beterraba	Cu, Pb, Zn	Ensaio em vaso, estufa *Lolium multiflorum* Lam
Solos contaminados pelo acidente da mina de Aznalcóllar[b]	Resíduo da extracção do azeite Composto de biossólidos Resíduo da fabricação de açúcar de beterraba	Cd, Cu, Mn, Zn	Ensaio em vaso em câmara de crescimento
Solos contaminados pelo acidente da mina de Aznalcóllar[c]	Estrume Composto Calcário	Cd,Cu, Fe,Mn, Pb, Zn	Ensaio de campo *Brassica juncea* (L.) Czern
Solos desenvolvido sobre *gossan* da mina de São Domingos[d]	Composto Polímero de poliacrilato	—	Ensaio em vaso, *outdoors* em condições controladas *Briza maxima* L. *Chaetopogon fasciculatus* (Link) Hayek *Spergularia purpurea* (Persoon) G. Don fil.
Solos desenvolvido sobre *gossan* da mina de São Domingos[e]	Composto de RSU	As, Ca, Fe, K, Mg, Na, P	Ensaio em vaso, *outdoors* em condições controladas *Dactylis glomerata* L. cv. Amba *Erica australis* L.
Diferentes solos contaminados (em laboratório) com lamas com pirite da mina de Aznalcóllar[f]	Carbonato de cálcio	Cu, Pb, Zn	Ensaio em vaso, incubação sob condições aeróbicas controlada
Solos contaminados pelo acidente da mina de Aznalcóllar[g]	Composto de biossólidos *Sugarbeet lime*	As,Ca, Cd, Cu,K, Mg, N, Pb, Pb, S, Zn	Ensaio em vaso, *outdoors* *Rosmarinus officinalis* L. (após crescimento de *A. stolonifera* e *C. dactilon* (L.) Pers)

Materiais a reabilitar	Correctivos	Elementos	Tipo de ensaio
Solos contaminados pelo acidente da mina de Aznalcóllar[h]	Resíduos florestais Leonardite *Sugarbeet lime* Composto de biossólidos Composto de RSU	Cd, Cu, Zn	Ensaio em vaso, *outdoors* *Agrostis stolonifera* L.
Solos contaminados pelo acidente da mina de Aznalcóllar[i]	*Sugarbeeet lime* Composto de biossólido Leonardite	As, Cd, Cu, Fe, Mn,Pb, Zn	Ensaio de campo com vegetação espontânea
Lamas com pirite da mina de Monte Romero[j]	Cinzas de biomassa e areia de quartzo	As, Ca, Cu, K, Fe, Mn, Na, Pb, Sb, SO_4, Zn	Ensaio de lixiviação em coluna, laboratório
Solo contaminado com adição de soluções de Ni, Pb e Zn[k]	Fosfogesso Gesso vermelho, Espuma de açúcar (*sugar foam*) Cinzas de biomassa	Ni, Pb, Zn	Ensaio em coluna, laboratório
Escombreira da mina Las Tórtolas[l]	Biossólidos	Cu, Cd, Mo, Zn,	Ensaio em vaso, estufa *Lolium perenne* L.
Solo contaminado com adição de doses crescente de Ni[m]	Composto de resíduos da cultura do algodão Estrume de aves	—	Ensaio em placa, câmara de incubação

[a]Alvarenga et al., 2008a; [b]Burgos et al. 2010; [c]Clemente et al., 2006; [d]de Varennes et al., 2009; [e]de Varennes et al., 2010; [f]Hinojosa et al., 2008; [g]Madejón et al., 2009; [h]Pérez-de-Mora et al., 2005; [i]Pérez-de-Mora et al., 2011; [j]Pérez-López et al., 2007; [k]Rodríguez-Jordá et al., 2010; [l]Santibáñez et al., 2008; [m]Tejada et al., 2008

A aplicação de compostos ou resíduos fosfatados (ex. farinha de osso) também é uma prática atractiva para imobilização de EPTs pois, pode promover a formação de algumas fases sólidas (ex. fosfatos de Pb, Zn ou U) bastante estáveis e de baixa solubilidade (Abreu et al., 2014a; Bolan et al., 2014; Kumpiene et al., 2008). Além disso, consoante a fonte, a aplicação de fosfato no solo pode causar adsorção directa dos catiões através do aumento da carga de superfície (Adriano et al., 2004).

De um modo geral, a aplicação de correctivos orgânicos ao solo melhora a fertilidade, a estrutura e a actividade microbiana bem como diminui a disponibilidade dos catiões devido à formação de complexos organo-metálicos, aumento da capacidade de troca catiónica ou das reacções redox (Adriano et al., 2004; Bolan et al., 2014; Burgos et al., 2010; Clemente et al., 2006; Tejada et al., 2008). A capacidade de imobilização depende do elemento, do tipo de solo e do tipo de matéria orgânica (Adriano et al., 2004; Bolan et al., 2014; Burgos et al., 2010; Kumpiene et al., 2008; Tejada et al., 2008). De facto, a aplicação de biossólidos frescos (ou sem prévia maturação), os quais apresentam geralmente baixo nível de humificação, contribuiu para a mobilização dos elementos químicos (Santibáñez et al., 2008).

A contribuição dos correctivos dependerá das suas características e do tipo de resposta face aos componentes do solo a reabilitar, pois os mesmos correctivos podem ter um efeito diferente consoante as características do solo (Burgo et al., 2010). Por outro lado, alguns correctivos, embora possam promover o desenvolvimento das plantas, aumentam o conteúdo de C orgânico dissolvido o que leva ao aumento da disponibilidade dos elementos e, consequentemente, à lixiviação dos mesmos (Santibáñez et al., 2008).

É de salientar ainda que, por vezes, pode não ocorrer a melhoria imediata de algumas propriedades dos solos/escombreiras. Hinojosa et al. (2008) observou um aumento da actividade enzimática após aplicação de *sugarbeeet lime* em solos contaminados, contudo a fracção disponível de Cu, Pb e Zn (extraída com DTPA) não mostrou diferenças. A mesma tendência foi indicada por de Varennes et al. (2010) onde a aplicação de composto a solos desenvolvidos sobre *gossan* aumentou a actividade enzimática enquanto as concentrações de Pb, Cu, Mn e Zn na fracção disponível não foram afectadas. Mesmo a nível biológico e tendo em conta que as respostas do microbiota são relativamente rápidas face à aplicação de correctivos orgânicos, por vezes o mau estado microbiológico do meio original implica primeiro o aumento da biomassa microbiana em vez da estimulação das funções bioquímicas (Burgo et al., 2010; Pérez-de-Mora et al., 2005).

Em locais com clima árido ou semiárido, o sucesso do estabelecimento de plantas, mesmo que tolerantes e autóctones, directamente nos solos a reabilitar pode ser um processo difícil. Pérez-de-Mora et al. (2011) verificou que a colonização natural (avaliada pelo grau de cobertura, biomassa produzida e riqueza específica de plantas vasculares) de solos contaminados pelo acidente da mina de Aznalcóllar foi mais rápida em *plots* com a aplicação de correctivos (Quadro 3). Por outro lado, em alguns tipos de escombreiras pode ser mesmo inviável (Tordoff et al., 2010).

Assim, a implementação do processo de fitoestabilização com adição de correctivos pode acelerar o processo de reabilitação de áreas contaminadas/degradas (Adriano et al., 2004; Favas et al., 2014; Freitas et al., 2004a). De Varennes et al. (2009, 2010) e Madejón et al. (2009) observaram uma melhoria mais significativa das características dos solos (ex. actividade enzimática, respiração basal,

biomassa microbiana, pH e/ou conteúdo de C orgânico) com a aplicação de resíduos orgânicos e/ou inorgânicos conjuntamente como crescimento de diferentes espécies (Quadro 3) comparativamente aos solos onde se fez unicamente aplicação de resíduos sem crescimento das plantas.

No entanto, é de realçar que o efeito dos resíduos, por aplicação individual ou em misturas, pode não se manter a médio-longo prazo pois, apenas alguns dos processos biogeoquímicos (ou propriedades dos solos/escombreiras) são melhorados. De facto, Pérez-de-Mora et al. (2011) indica que a aplicação repetida de correctivos pode ser necessária para a melhoria do desenvolvimento da vegetação e da redução da concentração dos EPTs nas plantas.

Assim, o sucesso da fitoestabilização das áreas mineiras a médio-longo prazo pode residir na concepção de um solo artificial derivado de resíduos (Tecnossolo – IUSS WorkingGroup WRB) de características adequadas ao objectivo da reabilitação ambiental (Tecnossolo "*à la carte*") que promova o crescimento de vegetação (Macías, 2004; Macías et al., 2007, 2011). Segundo estes autores, nos Tecnossolos são criados/estimulados diferentes processos biogeoquímicos e de pedogénese, devido aos efeitos complementares de cada componente, conduzindo assim à sustentabilidade do processo a médio-longo prazo. Esta condição ainda tem mais relevância para as áreas mineiras que apresentam uma contaminação multielementar e que geram drenagem ácida (Kumpiene et al., 2008) bem como aquelas que apresentam materiais de escombreira de granulometria grosseira ou muito fina. É de realçar ainda que, as perdas de C e N para a atmosfera pelos Tecnossolos são menores em comparação com a compostagem, devido à estabilização do C orgânico o que contribui para uma redução dos gases de efeito estufa e, consequentemente, da pegada ambiental do C (Macías, 2004; Macías e Arbestain, 2010).

O uso de Tecnossolos para recuperação de escombreiras de sulfuretos foi realizada com sucesso nas minas de As Pontes e Touro, ambas na Galiza − Espanha, onde se verificou uma diminuição da geração de drenagem ácida, com a consequente melhoria da qualidade das águas superficiais e subterrâneas, mas também da atividade biológica e promoção da biodiversidade (Macías et al., 2007; Monterroso et al., 1998).

Vários são os resíduos industriais ou agro-industriais que podem ser usados para a concepção de Tecnossolos, devido às suas características intrínsecas, grandes quantidades disponíveis e custo praticamente "zero" (muitas vezes só associado ao transporte), tendo deste modo um destino economicamente atractivo em vez de serem depositados em aterro (Macías, 2004; Macías et al., 2007, 2011). A selecção dos resíduos é um ponto fulcral no processo de concepção do Tecnossolo ao nível ambiental e económico pois, alguns resíduos contêm elevadas concentrações de elementos químicos ou precisam de um período de maturação (ex. lamas de ETAR ou composto).

1.6 CARACTERIZAÇÃO DE CISTUS LADANIFER E LAVANDULA PEDUNCULATA E SEUS PRODUTOS

O *Cistus ladanifer* L. (Fig. 5; vulgarmente designada por esteva) é um arbusto espontâneo da Família Cistaceae, fortemente aromático cujas partes aéreas são extremamente pegajosas. É encontrada em diferentes condições climáticas e tipos de solos de todo o mediterrâneo ocidental, desde Portugal e Marrocos até à Argélia, sendo muitas vezes a espécie dominante nos sistemas arbustivos mediterrâneos. Relativamente a Portugal Continental, esta planta aparece em quase todo o território tanto em áreas contaminadas como não contaminadas. Como outras espécies do género *Cistus* é uma planta pioneira bem adaptada a limitações nutricionais, hídricas e excesso de luz, bem como a outros factores de stresse usuais nos ecossistemas mediterrânicos como é o fogo (Correia, 2002; Delgado et al., 2001; Santos et al., 2013). É de salientar que dentro dos estádios de sucessão ecológica e de comportamento pioneiro, a esteva é muitas vezes a primeira espécie colonizadora.

Fig. 5 Planta de *Cistus ladanifer.*

Das espécies vegetais presentes nas áreas mineiras, a esteva é aquela que tem sido alvo de mais estudos (Alvarenga et al., 2004; Anawar et al., 2011; Batista et al., 2009; Durães et al., 2015; Favas et al., 2014; Freitas et al., 2004a; Kidd et al., 2004; Lázaro et al., 2006; Murciego et al., 2007; Pratas et al., 2005; Santos et al., 2009, 2012, 2013) apresentando uma vasta distribuição em diferentes tipos de escombreiras e solos. Como para outras espécies de *Cistus* (Abreu et al., 2012a,b; Favas et al.,

2014), o grau de tolerância e os padrões de acumulação dentro da planta face ao nível de contaminação parecem estar relacionados com a população (Batista et al., 2009; Kidd et al., 2004; Lázaro et al., 2006; Santos et al., 2009, 2012, 2013).

A tolerância e adaptabilidade de *C. ladanifer* aos factores de stresse ambiental e oxidativo estão associados a mecanismos específicos de conservação de água e nutrientes (Nuñes-Oliveira et al., 1996; Santos et al., 2013), dimorfismo das sementes e consequente variação germinativa (Delgado et al., 2001) bem como, restrição dos EPTs na raiz (Alvarenga et al., 2004; Batista et al., 2009; Kidd et al., 2004; Santos et al., 2012) e actividade de enzimas antioxidativas em diferentes locais das células das folhas (Santos et al., 2009). A taxa de crescimento desta espécie é elevada, quando as condições o permitem, produzindo considerável biomassa em relativamente pouco tempo (Nuñez et al., 1989; Patón et al., 1998).

A *Lavandula pedunculata* (Mill.) Cav. (sinónimos *Stoechas pedunculata* Mill, *L. pedunculata* subsp. s*ampaiana* (Rozeira) Franco; *L.. sampaiana* (Rozeira) Rivas Mart., TE Díaz & Fern. Gonz e *L. stoechas* subsp. *pedunculata* (Mill.) Rozeira) é uma das espécies aromáticas da Familia das Lamiaceae, vulgarmente conhecida por rosmaninho, lavanda ou lavandula (Fig. 6). É encontrada na Peninsula Ibérica, mais frequentemente para o interior, e Norte de Africa em diferentes habitats e tipos de solos.

Fig. 6 Planta de *Lavandula pedunculata.*

Esta espécie também foi identificada em algumas áreas mineiras (Pratas et al., 2005; Quental et al., 2002), nomeadamente em São Domingos (Freitas et al., 2004a), porém pouco é documentado

sobre esta planta em ambientes contaminados. Além disso, o conhecimento sobre o seu comportamento ecofisiológico em condições de contaminação é escasso.

Actualmente, os produtos derivados destas plantas aromáticas apresentam uma crescente tendência de mercado não só para a indústria de perfumaria/cosmética, farmacêutica (extracção de compostos bioactivos) e terapêutica (extractos, infusões, tinturas ou cápsulas) mas também para o sector industrial (exploração de tintas, corantes, produtos fitofarmacêuticos ou detergentes) (Lubbe e Verpoorte, 2011; Raut e Karuppayil, 2014). No entanto, o uso destas plantas em chá/infusões ou extractos, nomeadamente sob a forma de óleos, tinturas ou extractos aquosos, é uma prática antiga em sistemas tradicionais (WHO, 2007).

O *Cistus ladanifer* tem sido usado desde os tempos antigos, nomeadamente desde os egipcíos, devido ao seu exsudado aromático ou resina (vulgarmente designado como láudano), para tratar vários problemas de saúde (ex. diarreia, disenteria, catarro e desconforto da menstruação). Actualmente, o óleo de esteva (mas também o láudano) tem um elevado interesse para a indústria farmacêutica e perfumaria devido à sua propriedade fixadora de aromas e carácter anti-séptico, adstringente, tónico, expectorante e balsâmico (Barrajón-Catalán et al., 2010). De facto, extractos de folhas de esteva são uma fonte de distintos metabolitos secundários (ex. fenólicos, terpernos, ácidos gordos) com actividades antioxidantes e antimicrobianas (Andrade et al., 2009; Barrajón-Catalán et al., 2010) bem como fúngicas (Barros et al., 2013). Mesmo alguns estudos preliminares de actividade citotóxica de extractos aquosos de esteva em células cancerosas do pâncreas e mama têm mostrado algum potencial para suportar novos estudos (Barrajón-Catalán et al., 2010).

De igual modo, o uso de plantas do género *Lavandula* como um agente terapêutico remonta desde os romanos e gregos (Cavanagh e Wilkinson, 2002). Em Portugal, estudos etnobotânicos referem o uso de infusões de rosmaninho, para uso interno ou externo, devido às suas propriedades antissépticas, béquico, anti-inflamatório, anti-reumático, antiespasmódico, digestivo, diurético e calmante (Figueiredo et al., 2014). Cavanagh e Wilkinson (2002) também refere, para além das enunciadas, várias actividades biológicas de óleos de plantas do género *Lavandula*. A *Lavandula pedunculata* é também indicada como uma fonte de compostos naturais capazes de prevenir doenças neurodegenerativas (Costa et al., 2009) e com importante atividade antifúngica (Zuzarte et al., 2009). É de realçar que, na maioria das vezes, a bioactividade destes produtos pode estar associada a mais do que um componente do extracto, que pode não ser o maioritário (Bakkali et al., 2008).

De um modo geral, a produtividade e variabilidade fitoquímica dos produtos derivados de espécies aromáticas depende de vários factores associados às plantas (espécie, ecótipo ou idade da planta) e às condições ambientais e do meio onde as plantas se desenvolvem (Raut e Karuppayil, 2014). De facto, já foi observada variação na composição orgânica e, consequentemente, na actividade antioxidante segundo a espécie de *Lavandula* (Matos et al., 2009; Teixeira et al., 2013). González-Coloma et al. (2011) também verificaram que a concentração dos componentes maioritários de extractos de *Lavandula luisieri* (Rozeira) Rivas Mart. (extraídos com água e por hidrodestilação) variam consoante a população de plantas silvestres e condições de crescimento (silvestres *Vs* cultivadas). Ainda para *L. dentata* L., Masetto et al. (2011) concluiram que o teor de óleo extraído, por

hidrodestilação, e a composição orgânica variava com o estádio de desenvolvimento floral da espécie e a época de colheita.

A composição orgânica de extractos aquosos das folhas de plantas pertencentes ao género *Cistus* também está fortemente relacionada com o seu subgénero, porém as características do solo e o clima são factores que têm menor influência (Barrajón-Catalán et al., 2011).

Por outro lado, o rendimento e a composição dos extractos vegetais e, consequentemente, as suas propriedades biológicas variam com o material vegetal e o método de extracção (Raut e Karuppayil, 2014). González-Coloma et al. (2011) constataram que a composição maioritária de extractos de *L. luisieri* depende da parte da planta usada (flores ou folhas) na extracção.

Relativamente às metodologias, Andrade et al. (2009) verificaram que a extracção com acetona do óleo da parte aérea de *C. ladanifer* tinha um maior rendimento de extracção (14,19 %) e concentração total de compostos fenólicos (334,56 mg GAE/g extracto; GAE − *gallic acid equivalent*) e flavonóides (23,37 mg QE/g extracto; QE − *quercetin equivalent*), do que a realizada com etanol (8,49 %, 255,19 mg GAE/g extracto e 20,50 mg QE/g extracto). O rendimento e as propriedades antioxidantes de extractos metanólicos de *C. ladanifer* são ainda melhores do que os obtidos com acetona (Guimarães et al., 2010). Resultados semelhantes foram também obtidos para *L. pedunculata* onde o rendimento e a composição química varia consoante a solução extractante e, consequentemente, as suas propriedades antiradicais e antioxidantes (Costa et al., 2013; Teixeira et al., 2013).

Vários estudos têm sido realizados para a caracterização de extractos vegetais de *C. ladanifer* e das suas actividades biológicas e/ou potenciais aplicações industriais (Fernández-Arroyo et al., 2010; Guimarães et al., 2009, 2010; Verdeguer et al., 2011; Zidane et al., 2013). No entanto, os estudos para o género *Lavandula* resumem-se a extractos sob a forma de óleo essencial sendo que, em particular, para a espécie *L. pedunculata* os estudos são escassos (Costa et al., 2013; Matos et al., 2009; Teixeira et al., 2013; Zuzarte et al., 2009, 2010).

O conteúdo de elementos químicos, tanto nas matérias-primas vegetais como nos produtos derivados das plantas aromáticas/medicinais é um ponto importante a determinar já que podem ser considerados uma fonte de nutrientes mas, também de risco para a saúde pública. De facto, WHO (2007) editou uma série de *guidelines* para avaliação das plantas medicinais devido ao risco que existe para a saúde pública.

Embora Zheljazkov et al. (2008) tenham observado que altas concentrações de Cu, Cd e Pb no solo ou no meio de crescimento não conduzem à transferência dos mesmos elementos para o óleo essencial de várias espécies de plantas aromáticas (*Coriandrum sativum* L., *Anethum graveolens* L., *Chamomilla recutita* (L.) K., *Mentha x piperita* L., *Ocimum basilicum* L., *Hyssopus officinalis* L., *Melissa officinalis* L. e *Salvia officinalis* L.), nenhuma informação está disponível acerca da influência dos contaminantes do solo na composição e qualidade de produtos derivados de plantas de *C. ladanifer* e *L. pedunculata* (ou mesmo de espécies de plantas do mesmo género).

1.7 REFERÊNCIAS BIBLIOGRÁFICAS

Abreu, M.M., Magalhães, M.C.F., 2009. Phytostabilization of soils in mining areas. Case studies from Portugal. In: Aachen, L., Eichmann, P. (Eds.), Soil remediation. Nova Science Publishers, Inc. New York, pp. 297-344.

Abreu, M.M., Tavares, M.T., Batista, M.J., 2008. Potential use of *Erica andevalensis* and *Erica australis* in phytoremediation of sulphide mine environments: São Domingos, Portugal. Journal of Geochemical Exploration 96(2-3), 210-222.

Abreu, M.M., Santos, E.S., Magalhães, M.C.F., Nabais, C., 2009. Arsenic containing phases in soils from São Domingos mining area and in non-contaminated soils from Pomarão and Serra do Caldeirão. Revista de Ciências Agrárias XXX (1), 155-169.

Abreu, M.M., Batista, M.J., Magalhães, M.C.F., Matos, J.X., 2010a. Acid mine drainage in the Portuguese Iberian Pyrite Belt. In: Brock C.R. (Eds.), Mine drainage and related problems. Nova Science Publishers, Inc. New York, pp. 71-118.

Abreu M.M., Santos E.S., Ferreira M. B., Magalhães M.C.F., 2010b. Availability of Cu, Pb and Zn in Canal Caveira and São Domingos mine areas from Iberian Pyrite Belt. Revista de Ciências Agrárias, XXXIII(1), 358-366.

Abreu, M.M., Santos, E.S., Ferreira, M., Magalhães, M.C.F., 2012a. *Cistus salviifolius* a promising species for mine wastes remediation. Journal of Geochemical Exploration 113, 86-93.

Abreu, M.M., Santos, E.S., Fernandes, E., Magalhães M.C.F. 2012b. Trace elements tolerance, accumulation and translocation in *Cistus populifolius*, *Cistus salviifolius* and their hybrid growing in polymetallic contaminated mine areas. Journal of Geochemical Exploration 123, 52-60.

Abreu, M.M., Lopes, J., Santos, E.S., Magalhães, M.C.F., 2014a. Ecotoxicity evaluation of an amended soil contaminated with uranium and radium using sensitive plants. Journal of Geochemical Exploration 142, 112-121.

Abreu, M.M, Bech, J., Carvalho, L.C., Santos, E.S., 2014b. Potential hazardous elements fluxes from soil to plants and the food chain. In: Bini, C., Bech, J. (Eds.), PHEs, Environment and Human Health: Potentially harmful elements in the environment and the impact on human health. Springer, Netherlands, pp. 309-337.

Adriano, D.C., 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability and risk of metals. New York, Springer-Verlag.

Adriano, D.C., Wenzel, W.W., Vangronsveld, J., Bolan, N.S., 2004. Role of assisted natural attenuation in environmental cleanup. Geoderma 122, 121-142.

Ali, H., Khan, E., Sajad, M.A., 2013. Phytoremediation of heavy metals − Concepts and applications. Chemosphere 91 869-881.

Alvarenga, P.M., Araújo, M.F., Silva, J.A.L., 2004. Elemental uptake and root leaves transfer in *Cistus ladanifer* L. growing in a contaminated pyrite mining area (Aljustrel-Portugal). Water, Air and Soil Pollution 152, 81-96.

Alvarenga, P., Palma, P., Gonçalves, A.P., Baião, N., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008a. Assessment of chemical, biochemical and ecotoxicological aspects in a mine soil amended with sludge of either urban or industrial origin. Chemosphere 72, 1774-1781.

Alvarenga, P., Palma, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008b. Evaluation of tests to assess the quality of mine-contaminated soils. Environmental Geochemistry and Health 30, 95-99.

Alvarenga, P., Palma, P., de Varennes, A., Cunha-Queda, A.C., 2012. A contribution towards the risk assessment of soils from the São Domingos Mine (Portugal): chemical, microbial and ecotoxicological indicators. Environmental Pollution 161, 50-56.

Alvarenga, P., Simões, I., Palma, P., Amaral, O., Matos, J.X., 2014. Field study on the accumulation of trace elements by vegetables produced in the vicinity of abandoned pyrite mines. Science of the Total Environment 470-471, 1233-1242.

Álvarez-Valero, A.M., Pérez-López, R., Matos, J., Capitán, M.A., Nieto, J.M., Sáez, R., Delgado, J., Caraballo, M., 2008. Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian Peninsula): evidence from a chemical and mineralogical characterization. Environmental Geology 55(8), 1797-1809.

Álvarez-Valero, A.M., Sáez, R., Pérez-López, R., Delgado, J., Nieto, J.M., 2009. Evaluation of heavy metal bio-availability from Almagrera pyrite-rich tailings dam (Iberian Pyrite Belt, SW Spain) based on a sequential extraction procedure. Journal of Geochemical Exploration 102, 87-94.

Anawar, H.M., Freitas, M.C., Canha, N., Regina, I.S., 2011. Arsenic, antimony, and other trace element contamination in a mine tailings affected area and uptake by tolerant plant species. Environmental Geochemistry and Health 33, 353-362.

Andrade, D., Gil, C., Breitenfeld, L., Domingues, F., Duarte, A.P., 2009. Bioactive extracts from *Cistus ladanifer* and *Arbutus unedo* L.. Industrial Crops and Products 30, 165-167.

Anjos, C., Magalhães, M.C.F., Abreu, M.M., 2012. Metal (Al, Mn, Pb and Zn) soils extractable reagents for available fraction assessment: Comparison using plants, and dry and moist soils from the Braçal abandoned lead mine area, Portugal. Journal of Geochemical Exploration 113, 45-55.

Araújo, A.S.F., Monteiro, R.R.T., 2007. Indicadores biológicos de qualidade do solo. Bioscience Journal 23(3), 66-75.

Asta, M.P., Cama, J., Gault, A.G., Charnock, J.M., Queralt, I. 2007. Characterization of and sediments in the discharge of the Tinto Santa Rosa Mine (Iberian Pyritic Belt, SW Spain). In: Cidu, R., Frau, F. (Eds.), IMWA Symposium 2007: Water in Mining Environments, 5 pp.

Ávila, P.F., Ferreira da Silva, E., Salgueiro, A.R., Farinha, J.A., 2008. Geochemistry and mineralogy of mill tailings impoundments from the Panasqueira Mine (Portugal): Implications for the surrounding environment. Mine Water and the Environment 27, 210-224.

Baker, A.J.M., 1981. Accumulators and excluders strategies in the response of plants to heavy metals. Journal of Plant Nutrition 3, 643-654.

Baker, A.J.M., Walker, P.L., 1990. Eco-physiology of metal uptake by tolerant plants, CRC Press, Boca Raton, pp. 155-178.

Bandick, A.K., Dick, R.P., 1999. Field management effects on soil enzyme activities. Soil Biology and Biochemistry 31, 1471-1479.

Barceló, J., Poschenrieder, C., 2003. Phytoremediation: principles and perspectives. Contributions to Science 2(3), 333-344.

Barrajón-Catalán, E., Fernández-Arroyo, S., Saura, D., Guillén, E., Fernández-Gutiérrez, A., Segura-Carretero, A., Micol, V., 2010. Cistaceae aqueous extracts containing ellagitannins show antioxidant andantimicrobial capacity and cytotoxic activity against human cancer cells. Food and Chemical Toxicology 48, 2273-2282.

Barriga, F.J.A.S., Carvalho, D., Ribeiro, A., 1997. Introduction to the Iberian Pyrite Belt. SEG Neves Corvo Field Conference Guidebook 27, 1-20.

Barros, L., Dueñas, M., Alves, C.T., Silva, S., Henriques, M., Santos-Buelga C., Ferreira, I.C.F.R., 2013. Antifungal activity and detailed chemical characterization of *Cistus ladanifer* phenolic extracts. Industrial Crops and Products 41, 41-45.

Batista, M.J., González-Fernández, O., Abreu, M.M., Carvalho, L., 2009. Chemical elements variation in leaves with different development stages of *Cistus* plants from S. Domingos mine area, South Portugal. Proceedings

VII Congresso Ibérico e X Congresso Nacional de Geoquímica 407-415. Disponível em: http:// repositorio.lneg.pt/bitstream/10400.9/740/1/33810.pdf. Consultado a Junho 2015

Bech, J., Abreu, M.M., Chon, H.T., Roca, N. 2014. Remediation of potentially toxic elements in contaminated soils. In: Bini, C., Bech, J. (Eds.), PHEs, environment and human health: potentially harmful elements in the environment and the impact on human health. Springer, Netherlands, pp. 253-308

Berti, W.R., Jacobs, L.W., 1996. Chemistry and phytotoxicity of soil trace elements from repeated sewage sludge application. Journal of Environmental Quality 25, 1025-1032 .

Bleeker, P.M., Assunção, A.G.L., Teiga, P.M., Koe, T., Verkleij, J.A.C., 2002., Revegetation of acidic, as-contaminated Jales mine spoil tips using a combination of spoil amendments and tolerant grasses. The Science of the Total Environment 300, 1-13.

Belyaeva, O.N., Haynes, R.J., Birukova, O.A., 2005. Barley yield and soil microbial and enzyme activities as affected by contamination of two soils with lead, zinc or copper. Biology and Fertility of Soils 41, 85-94.

Bienfait, H.F., Duivenvoorden, J., Verkerke, W., 1982. Ferric reduction by roots of chlorotic bean plants: indications for enzymatic process. Journal of Plant Nutrition 5, 451-456. In: Lasat, M.M., 2002. Phytoextraction of toxic metals: a review of biological mechanisms. Journal of Environmental Quality 31, 109-120.

Bolan, N., Kunhikrishnan, A., Thangarajan, R., Kumpiene, J., Park, J., Makino, T., Kirkham, M.B., Scheckel, K., 2014. Remediation of heavy metal(loid)s contaminated soils – To mobilize or to immobilize?. Journal of Hazardous Materials 266,141-166.

Bourrelier, P.H., Berthelin, J., Pédro, G., 1998. Dynamique des éléments en traces dans les sols : mobilité et transferts vers les eaux souterraines et les plantes, contamination des sols par le éléments en traces : les risques et leur gestion. Académie des Sciences 42, Lavoisier Tec&Doc., Paris, 440.

Brümmer, G.W., 1986. Heavy metal species, mobility and availability in soils. In: Bernhard, M., Brinckman, F.E., Sadler, P.J. (Eds.), The importance of chemical “speciation” in environmental processes. Springer-Verlang, Berlin, pp. 169-192.

Burgos, P., Madejón, P., Cabrera, F., Madejón, E., 2010. By-products as amendment to improve biochemical properties of trace element contaminated soils: effects in time. International Biodeterioration and Biodegradation 64, 481-488.

Cabezudo, B., Rivera, J., 1980. Notas taxonómicas y corológicas sobre la Flora de Andalucía Occidental. 2: *Erica andevalensis* Cabezudo & Rivera sp. nov. Lagascalia 9 (2), 223-226.

Cao, X., Ma, L.Q., Tu, C., 2004.Antioxidative responses to arsenic in the arsenic-hyperaccumulator chinese brake fern (Pteris *vittata* L.). Environmental Pollution 128, 317-325.

Cavanagh, H.M.A., Wilkinson, J.M., 2002. Biological activities of lavender essential oil. Phytotherapy Research 16, 301-308.

CCME − Canada Council of Ministers of the Environment, 2007. Canadian soil quality guidelines for the protection of environmental and human health: summary tables (Updated September, 2007). CCME, Winnipeg, Canada.

Clemente, R., Concepción, A., Bernal, M.P., 2006. A remediation strategy based on active phytoremediation followed by natural attenuation in a soil contaminated by pyrite waste. Environmental Pollution 143(3), 397-406.

Conesa, H.M., Faz, Á., Arnaldos, R., 2006. Heavy metal accumulation and tolerance in plants from mine tailings of the semiarid Cartagena − La Unión mining district (SE Spain). Science of the Total Environment 366, 1-11.

Correia, O., 2002. Os *Cistus*: as Espécies do Futuro?. In: Loução, K.A. (Ed.) Fragmentos de ecologia. Escolar Editora, Portugal, pp. 97-119.

Costa, P., Gonçalves, S., Valentão, P., Andrade, P.B., Almeida, C., Nogueira, J.M.F., Romano, A., 2013. Metabolic profile and biological activities of *Lavandula pedunculata* subsp. *lusitanica* (Chaytor) Franco: Studies on the essential oil and polar extracts. Food Chemistry 141, 2501-2506.

de la Fuente, V., Rufo, L., Rodríguez, N., Amils, R., Zuluaga, J., 2010. Metal accumulation screening of the Río Tinto Flora (Huelva, Spain). Biological Trace Element Research 134, 318-341.

de Varennes, A., Cunha-Queda, C., Qu, G., 2009. Amendment of an acid mine soil with compost and polyacrylate polymers enhances enzymatic activities but may change the distribution of plant species. Water, Air and Soil Pollution 208(1), 91-100.

de Varennes, A., Abreu, M.M., Qum G., Cunha-Queda, C., 2010. Enzymatic activity of a mine soil varies according to vegetation cover and level of compost applied. International Journal of Phytoremediation 12(4), 371-383.

Delgado, J.A., Serrano, J.M., López, F., Acosta, F.J., 2001. Heat shock, mass-dependent germination, and seed yield as related components of fitness in *Cistus ladanifer*. Environmental and Experimental Botany 46, 11-20.

Dinelli, E., Lombini, A., 1996. Metal distributions in plants growing on copper mine spoils in northern Apennines, Italy: the evaluation of seasonal variations. Applied Geochemistry 11, 375-385.

Ernst, W.H.O., 1996. Bioavailabity of heavy metals and decontamination of soils by plants. Applied Geochemistry 11, 163-167.

Favas, P.J.C., Pratas, J., Varun, M., D'Souza, R., Paul, M.S., 2014. Phytoremediation of soils contaminated with metals and metalloids at mining areas: potential of native flora. In: Hernandez-Soriano, M.C., (Ed.), Environmental risk assessment of soil contamination. InTech, pp. 485-517.

Feng, M., Shan, X., Zhang, S., Wen, B., 2005. A comparison of rhizosphere-based method with DTPA, EDTA, $CaCl_2$ and NaNO3 extraction methods for prediction of bioavailability of metals in soil to barley. Environmental Pollution 137, 231-240.

Ferreira da Silva, E., Durães, N., Reis, P., Patinha, C., Matos, J., Costa, M.R., 2015. An integrative assessment of environmental degradation of Caveira abandoned mine area (Southern Portugal). Journal of Geochemical Exploration 159, 33-47.

Figueiredo, A.C., Pedro, L.G., Barroso, J.G., Trindade, H., Sanches, J., Oliveira, C., Correia, M., 2014. *Lavandula luisieri* (Rozeira) Rivas-Martínez e *Lavandula pedunculata* (Mill.) Cav.. Revista Agrotec 14, 38-41.

Freitas, H., Prasad, M.N.V., Pratas, J., 2004a. Plant community tolerant to trace elements growing on degraded soils of São Domingos mine in the south east of Portugal: environmental implications. Environment International 30, 65-72.

Freitas, H., Prasad, M.N.V., Pratas, J., 2004b. Analysis of serpentinophytes from north-east of Portugal for trace metal accumulation − relevance to the management of mine environment. Chemosphere 54, 1625-1642.

Freitas, M.C., Pacheco, A.M.G., Anawar, H.M., Dionísio, I., Dung, H.M., Canha, N., Bettencourt, A., Henriques, F., Pinto-Gomes, C.J., Capelo, S., 2009. Determination of phytoextraction potential of plant species for toxic elements in soils of abandoned sulphide-mining areas. ournal of Radioanalytical and Nuclear Chemistry 282, 21-27.

García-Lorenzo, M.L., Martínez-Sánchez, M.J., Pérez-Sirvent, C., Molina, J., 2009. Ecotoxicological evaluation for the screening of areas polluted by mining activities. Ecotoxicology 18, 1077-1086.

Ghosh, M., Singh, S.P., 2005. A review on phytoremediation of heavy metals and utilization of it's by products. Asian Journal on Energy and Environment 6(4), 214-231.

González-Coloma, A., Delgado, F., Rodilla, J.M., Silva, L., Sanz, J,, Burillo, J., 2011. Chemical and biological profiles of *Lavandula luisieri* essential oils from western Iberia Peninsula populations. Biochemical Systematics and Ecology 39, 1-8.

González-Fernández, O., Batista, M.J., Abreu, M.M., Queralta, I., Carvalho, M.L. 2011. Elemental characterization of edible plants and soils in an abandoned mining region: assessment of environmental risk. X-Ray Spectrometry 40, 353-363.

Grant, J.J., Loake, G.J., 2000. Role of active oxygen intermediates and cognate redox signalling in disease resistance. Plant Physiology 124, 21-29.

Guimarães, R., Barros, L., Carvalho, A.M., Sousa, M.J., Morais, J.S., Ferreira, IC.F.R., 2009. Aromatic plants as a source of important phytochemicals: Vitamins, sugars and fatty acids in *Cistus ladanifer*, *Cupressus lusitanica* and *Eucalyptus gunnii* leaves. Industrial Crops and Products 30, 427-430.

Guimarães, R., Sousa, M.J., Ferreira, I.C.F.R., 2010. Contribution of essential oils and phenolics to the antioxidant properties of aromatic plants. Industrial Crops and Products 32, 152-156.

Hinojosa, M.B., Carreira, J.A., Rodríguez-Maroto, J.M., García-Ruíz, R., 2008. Effects of pyrite sludge pollution on soil enzyme activities: ecological dose–response model. Science of the Total Environment 396, 89-99.

Hinsinger, P., Plassard, C., Jaillard, B., 2006. Rhizosphere: A new frontier for soil biogeochemistry. Journal of Geochemical Exploration 88, 210-213.

IUSS Working Group WRB, 2007. World reference base for soil resources 2006 (first update 2007). World Soil Resources Reports No. 103, Rome, FAO.

Kabata-Pendias, A., 2004. Soil-plant transfer of trace elements-an environmental issue. Geoderma 122, 143-149.

Kabata-Pendias, A., 2011. Trace elements in soils and plants. CRC Press, Boca Raton.

Khan, F.I., Husain, T., Hejazi, R., 2004. An overview and analysis of site remediation technologies. Journal of Environment Management 71, 95-122.

Kidd, P.S., Díez, J., Monterroso, C.M., 2004. Tolerance and bioaccumulation of heavy metals in five populations of *Cistus ladanifer* L. subsp. *ladanifer*. Plant and Soil 258, 189-205.

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments − a review. Waste Management 28, 215-225.

Lázaro, J.D., Kidd, P.S., Martínez, C.M., 2006. A phytogeochemical study of the Trás-os-Montes region (NE Portugal): possible species for plant-based soil remediation technologies. Science of the Total Environment 354, 265-277.

Levitt, J., 1980. Responses of plants to environmental stresses, Academic Press, Nova York. In: Ross, S., 1994, Toxic metals in soil-plant systems. Wiley publishers, Bristol.

Lindsay, W.L., Norvell, W.A., 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Science 42, 421-428.

Lubbe, A., Verpoorte, R., 2011. Cultivation of medicinal and aromatic plants for specialty industrial materials. Industrial Crops and Products 34, 785-801.

Macías, F., 2004. Recuperación de suelos degradados, reutilización de resíduos y secuestro de carbono. Una alternativa integral de mejora de la calidad ambiental. Recursos Rurais 1, 49-56.

Macías, F., Arbestain, M.C., 2010. Soil carbon sequestration in a changing global environment. Mitigation and Adaptation Strategies for Global Change 15(6), 511-529.

Macías, F., Bao, M., Macias-Garcia, F., Arbestain, M.C., 2007. Valorización biogeoquímica de Residuos mediante la elaboración de Tecnosoles con diferentes aplicaciones ambientales. Aguas & Residuos, série III, 5, 12-25.

Macías, F., Macías-García, F., Nieto, C., Verde, J.R., Pérez, C., Bao, M., Camps-Arbestain, M., 2011. Gestión de residuos y cambio climático. In: Mosquera, M.E.L., Osés, M.J.S., (Eds.), Gestión de residuos orgánicos de uso agrícola. Servizo de Publicacións e Intercambio Científico de la Universidade de Santiago de Compostela, Santiago de Compostela.

Madejón, P., Burgos P., Cabrera F., Madejón E. 2009. Phytostabilization of amended soils polluted with trace elements using the Mediterranean shrub: *Rosmarinus officinalis*. International Journal of Phytoremediation 11, 542-557.

Márquez-García, B., Córdoba, F., 2009. Antioxidative system and oxidative stress markers in wild populations of *Erica australis* L. differentially exposed to pyrite mining activities. Environmental Research 109, 968-974.

Martínez-Salgado, M.M., Gutiérrez-Romero, V., Jannsens, M., Ortega-Blu, R., 2010. Biological soil quality indicators: a review. In: Mendez-Vilas A. (Ed.) Current research, technology and education topics in applied microbiology and microbial biotechnology. FORMATEX Microbiology Series number 2, 1 pp. 319-328.

Martins, M., Santos, E.S., Faleiro, M.L., Chaves, S., Tenreiro, R., Barros, R.J., Barreiros, A., Costa, M.C. 2011. Performance and bacterial community shifts during bioremediation of acid mine drainage from two Portuguese mines. International Biodeterioration & Biodegradation 65, 972-981.

Masetto, M.A.M. Deschamps, C., Mógor, A.F., Bizzo, H.R., 2011. Teor e composição do óleo essencial de inflorescências e folhas de *Lavandula dentata* L. em diferentes estádios de desenvolvimento floral e épocas de colheita. Revista Brasileira de Plantas Medicinais 13(4), 413-421.

Matos, F., Miguel, M.G., Duarte, J., Venâncio, F., Moiteiro, C., Correia, A.I.D., 2009. Antioxidant capacity of the essential oils from *Lavandula luisieri*, *L. stoechas* subsp. *lusitanica*, *L. stoechas* subsp. *lusitanica L. luisieri* and *L. viridis* grown in Algarve (Portugal). Journal of Essential Oil Research 21, 327-336.

Matos, J.X., Martins, L., 2003. Itinerários geo–eco–educacionais como factor de desenvolvimento sustentado do turismo temático associado à Faixa Piritosa Ibérica. Actas IV Congreso Internacional do Património Geológico y Minero, 539-557.

Matos, J.X., Martins, L.P., 2006. Reabilitação ambiental de áreas mineiras do sector português da Faixa Piritosa Ibérica: estado da arte e perspectivas futuras. Boletín Geológico y Minero 117, 289-304.

Matos, J.X., Pereira, Z., Oliveira, V., Oliveira, J.T., 2006. The geological setting of the São Domingos pyrite orebody, Iberian Pyrite Belt. Actas do VII Congresso Nacional de Geologia, 283-286.

McBride, M.B., 1989. Reactions controlling heavy metal solubility in soils. Advances in Soil Sciences 10, 1-56. In: Prasad M.N., Hagemeyer, J., (Eds.), Heavy metal stress in plants from molecules to ecosystems. Springer, Berlim.

McLaughlin, M.J., Zarcinas, B.A., Stevens, D.P., Cook, N., 2000. Soil testing for heavy metals. Communications in Soil Science and Plant Analysis 31 (11), 1661-1700.

Meers, E., Samson, R., Tack, F.M.G., Ruttens, A., Vandegehuchte, M., Vangronsveld, J., Verloo, M.G., 2007. Phytoavailability assessment of heavy metals in soils by single extractions and accumulation by *Phaseolus vulgaris*. Environmental and Experimental Botany 60, 385-396,

Mendez, M.O., Maier, R.M., 2008. Phytoremediation of mine tailings in temperate and arid environments. Reviews in Environmental Science and Biotechnology 7, 47-59.

Mingorance, M.D., Leidi, E.O., Valdés, B., Rosini Oliva, S., 2012. Evaluation of lead toxicity in *Erica andevalensis* as an alternative species for revegetation of contaminated soils. International Journal of Phytoremediation 14(2), 174-185.

Monterroso, C., Macías, F., Gil Bueno, A., Val Caballero, C., 1998. Evaluation of the land reclamation project at the As Pontes Mine (NW Spain) in relation to the suitability of the soil for plant growth. Land Degradation & Development 9, 441-451.

Murciego, A.M., Sánchez, A.G., González, M.A.R., Gil, E.P., Gordillo, C.T., Fernández, J.C., Triguero, T.B., 2007. Antimony distribution and mobility in topsoils and plants (*Cytisus striatus*, *Cistus ladanifer* and *Dittrichia viscosa*) from polluted Sb-mining areas in Extremadura (Spain). Environmental Pollution 145, 15-21.

Nadgórska-Socha, A., Ptasiński, B., Kita, A., 2013. Heavy metal bioaccumulation and antioxidative responses in *Cardaminopsis arenosa* and *Plantago lanceolata* leaves from metalliferous and non-metalliferous sites: a field study. Ecotoxicology 22, 1422-1434.

NCR − National Research Council, 2005. Mineral tolerance of animals. National Academies Press, Washington.

Núñez, E., Cabeza. J., Escudero, J.C., 1989. Relación entre la biomasa de jarales y su rendimiento energetico por pirolisis. Options Méditerranéennes 3, 345-350.

Núñez-Olivera, E., Martínez-Abaigar, J., Escudero, J.C., 1996. Adaptability of leaves of *Cistus ladanifer* to widely varying environmental conditions. Functional Ecology 10, 636-646.

Oliveira, J.M.S., Farinha, J., Matos, J.X., Ávila, P., Rosa, C., Machado, M.J.C., Daniel, F.S., Martins, L., Leite, M.R.M., 2002. Diagnóstico Ambiental das Principais Áreas Mineiras Degradadas do País. Boletim Minas IGM 39(2), 67-85.

Ortiz, M. 2003. Aproximación a la minería y metalurgia de minas de Riotinto desde la antigüedad al siglo XIX. Tese de Doutoramento. Universidad Politécnica de Madrid, Espanha.

Pang, J., Chan, G.S.Y., Zhang, J., Liang, J., Wong, M.H., 2003. Physiological aspects of vetiver grass for rehabilitation in abandoned metalliferous mine wastes. Chemosphere 52, 1559-1570.

Patón, D., Azocar, P., Tovar, J., 1998., Growth and productivity in forage biomass in relation to the age assessed by dendrochronology in the evergreen shrub *Cistus ladanifer* (L.) using different regression models. Journal of Arid Environments 38, 221-235.

Pérez-de-Mora, A., Ortega-Calvo, J.J., Cabrera, F., Madejón, E., 2005. Changes in enzyme activities and microbial biomass after "*in situ*" remediation of a heavy metal-contaminated soil. Applied Soil Ecology 28, 125-137.

Pérez-de-Mora, A., Madejón, P., Burgos, P., Cabrera, F., Lepp, N.W., Madejón, E., 2011. Phytostabilization of semiarid soils residually contaminated with trace elements using by-products: Sustainability and risks. Environmental Pollution 159, 3018-3027.

Pérez-López, R., Nieto, J.M., Almodóvar, G.R., 2007. Immobilization of toxic elements in mine residues derived from mining activities in the Iberian Pyrite Belt (SW Spain): Laboratory experiments. Applied Geochemistry 22, 1919-1935.

Pérez-López, R., Álvarez-Valero, A.M., Nieto, J.M., Sáez, R., Matos, J.X., 2008. Use of sequential extraction procedure for assessing the environmental impact at regional scale of the São Domingos Mine (Iberian Pyrite Belt). Applied Geochemistry 23, 3452-3463.

Pérez-López, R., Márquez-García, B., Abreu, M.M., Nieto, J.M., Córdoba, F., 2014. *Erica andevalensis* and *Erica australis* growing in the same extreme environments: Phytostabilization potential of mining areas. Geoderma 230-231, 194-203.

Pratas, J., Prasad, M.N.V., Freitas, H., Conde, L., 2005. Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation. Journal of Geochemical Exploration 85, 99-107.

Quental, L., Bourguignon, A., Sousa, A.J., Batista, M.J., Brito, M.G., Tavares, T., Abreu, M.M., Vairinho, M., Cottard, F., 2002. MINEO Southern Europe environment test site. Contamination impact mapping and modelling - Final Report. Assessing and monitoring the environmental impact of mining activities in Europe using advanced Earth Observation Techniques (MINEO) 5yth FP-IST-1999-10337

Rauret, G., López-Sánchez, J.F., Sahuquillo, A., Rubio, R., Davidson, C., Ureb, A., Quevauviller, Ph., 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil referent materials. Journal of Environmental Monitoring 1, 57-61.

Raut, J.S., Karuppayil, M. 2014. A status review on the medicinal properties of essential oils. Industrial Crops and Products 62, 250-264.

Rodríguez-Jordá, M.P., Garrido, F., García-González, M.T., 2010. Potential use of gypsum and lime rich industrial by-products for induced reduction of Pb, Zn and Ni leachability in an acid soil. Journal of Hazardous Materials 175, 762-769.

Rosini Oliva, S., Valdés, B., Leidi, E.O., 2009. Accumulation and in vivo tissue distribution of pollutant elements in *Erica andevalensis*. Science of the Total Environment 407, 1929-1936.

Sáez, R., Pascual, E., Toscano, M., Almodovar, G.R., 1999. The Iberian type of volcano-sedimentary massive sulphide deposits. Mineralium Deposita 34, 549-570.

Sáinz, A., Grande, J.A., de la Torre, M.L., 2003. Analysis of the impact of local corrective measures on the input of contaminants from the Odiel River to the Ría of Huelva (Spain). Water, Air and Soil Pollution 144, 375-389.

Sánchez-España, J., 2008. Acid mine drainage in the Iberian Pyrite Belt: an overview with special emphasis on generation mechanisms, aqueous composition and associated mineral phases. Macla 10, 34-43.

Sánchez-España, J., Pamo, E.L., Santofimia, E., Aduvire, O., Reyes, J.A, Barettino, D., 2005. Acid mine drainage in the Iberian Pyrite Belt (Odiel river watershed, Huelva, SW Spain): Geochemistry, mineralogy and environmental implications. Applied Geochemistry 20(7), 1320-1356.

Sánchez-España, J., Pamo, E.L., Santofimia, P.E., Reyes, J.A., Martín, J.A.R., 2006., The removal of dissolved metals by hydroxysulphate precipitates during oxidation and neutralization of acid mine waters, Iberian Pyrite Belt. Aquatic Geochemistry 12, 269-298.

Sánchez-España, J., Toril, G.E., Pamo, E.L., Amils, R., Ercilla, M.D., Pastor, E.S., San Martín-Úriz, P., 2008. Biogeochemistry of hyperacid and ultraconcentrated pyrite leachate in San Telmo mine (Iberian Pyrte Belt, Spain). Water, Air and Soil Pollution 194, 243-257.

Santibáñez, C., Verdugo, C., Ginocchio, R., 2008. Phytostabilization of copper mine tailings with biosolids: Implications for metal uptake and productivity of Lolium perenne. Science of the Total Environment 395, 1-10.

Santos, E.S., Abreu, M.M., Nabais, C., Saraiva, J., 2009. Trace elements and activity of antioxidative enzymes in *Cistus ladanifer* L. growing on an abandoned mine area. Ecotoxicology 18, 860-868.

Santos, E.S., Abreu, M.M., Nabais, C., Magalhães, M.C.F., 2012. Trace element distribution in soils developed on *gossan* mine wastes and *Cistus ladanifer* L. tolerance and bioaccumulation. Journal of Geochemical Exploration 123, 45-51.

Santos, E.S., Nabais, C., Abreu, M.M., 2013. Adaptabilidade ecofisiológica de diferentes populações de *Cistus ladanifer* L. do sul de Portugal. STUDIA – Scientiæ Rerum Diffusio: Suplemento Temático IV Seminário Luso-brasileiro em Ciências do Ambiente e Empresariais, 6 pp

Schollenberger, C.J., Simon, R.H., 1945. Determination of exchange capacity and exchangeable bases in soil-ammonium acetate method. Soil Science 59, 13-24.

Sharma, P., Dubey, R.S., 2005. Lead toxicity in plants. Brazilian Journal of Plant Physiology 17, 35-52.

Sierra, M.J., Millán, R., Esteban, E., 2009. Mercury uptake and distribution in *Lavandula stoechas* plants grown in soil from Almadén mining district (Spain). Food and Chemical Toxicology 47, 2761-2767.

Słomka, A., Libik-Koniecznyb, M., Kutaa, E., Miszalskib, Z., 2008. Metalliferous and non-metalliferous populations of *Viola tricolor* represent similar mode of antioxidative response. Journal of Plant Physiology 165, 1610-1619.

Rocha. L., Rodrigues. S.M., Lopes. I., Soares. A.M.V.M., Duarte. A.C., Pereira. E., 2011. The water-soluble fraction of potentially toxic elements in contaminated soils: Relationships between ecotoxicity, solubility and geochemical reactivity. Chemosphere 84, 1495-1505.

Rodríguez-Jordá, M.P., Garrido, F., García-González, M.T., 2010. Potential use of gypsum and lime rich industrial by-products for induced reduction of Pb, Zn and Ni leachability in an acid soil. Journal of Hazardous Materials 175, 762-769.

Romero, F.M., Prol-Ledesma, R.M., Canet, C., Alvares, L.N., Pérez-Vázquez, R., 2010. Acid drainage at the inactive Santa Lucia mine, western Cuba: Natural attenuation of arsenic, barium and lead, and geochemical behavior of rare earth elements Applied Geochemistry 25, 716-727.

Tang, Y.T, Deng, T.H.B, Wu, Q.H., Wang, S.Z., Qiu, R.L., Wei, Z.B., Guo, X.F., Wu, Q.T, Lei, M., Chen, T.B., Echevarria, G., Sterckeman, T., Simonnot, M.O., Morelm J.L., 2012. Designing cropping systems for metal-contaminated sites: a review. Pedosphere 22(4), 470-488.

Teklić, T., Hancock, J.T., Engler, M., Paradicović, N., Cesar, V., Lepeduš, H., Štolfa, I., Bešlo, D., 2008.Antioxidative responses in radish (*Raphanus sativus* L.) plants stressed by copper and lead in nutrient solution and soil. Acta Biologica Cracoviensia Series Botanica 50(2), 79-86.

Teixeira, S., Mendes, A., Alves, A., Santos, L., 2007. Simultaneous distillation-extraction of high volatile compounds from *Cistus ladanifer* L.. Analytica Chimica Acta 584, 439-446.

Tejada, M., Moreno, J.L.,. Hernández, M.T, García, C., 2008. Soil amendments with organic wastes reduce the toxicity of nickel to soil enzyme activities. European Journal of Soil Biology 44, 129-140.

Tessier, A., Campbell, P.G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation particulate trace metals. Analytical Chemistry 51, 844-854.

Tordoff, G.M., Baker, A.J.M., Willis, A.J., 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere 41, 219-228.

Tornos, F., 2006. Environment of formation and styles of volcanogenic massive sulfides: The Iberian Pyrite Belt. Ore Geology Reviews 28, 259-307.

Trigueros, D., Mingorance, M.D., Rossini Oliva, S., 2012. Evaluation of the ability of *Nerium oleander* L. to remediate Pb-contaminated soils. Journal of Geochemical Exploration 114, 126-133.

.Valente, T.M., Gomes, C.L., 2009. Occurrence, properties and pollution potential of environmental minerals in acid mine drainage. Science of the Total Environment 407(3), 1135-1152.

Verdeguer, M., Blázquez, M.A., Boira, H., 2011. Chemical composition and herbicidal activity of the essential oil from a *Cistus ladanifer* L. population from Spain. Natural Product Research 6(17), 1602-1609.

Wang, X., Shan, X., Zhang, S., Wen, B., 2004. A model for evaluation of the phytoavailability of trace elements to vegetables under the field conditions. Chemosphere 55, 811-822.

WHO − World Health Organization 2007. Guidelines for assessing quality of herbal medicines with reference to contaminants and residues. World Health Organization.

Wong, M.H., 2003. Ecological restoration of mine degraded soils with emphasis on metal contaminated soils. Chemosphere 50, 775-780.

Zheljazkov, V.D., Craker, L.E., Xing, B., Nielsen, N.E., Wilcox, A., 2008. Aromatic plant production on metal contaminated soils. Science of the Total Environment 395, 51-62.

Zidane, H., Elmiz, M., Aouinti, F., Tahani, A., Wathelet, J., Sindic, M., Elbachiri, A., 2013. Chemical composition and antioxidant activity of essential oil, various organic extracts of *Cistus ladanifer* and *Cistus libanotis* growing in Eastern Morocco. African Journal of Biotechnology 12(34), 5314-5320.

Zitka, O., Krystofova, O., Hynek, D., Sobrova, P., Kaiser, J., Sochor, J., Zehnalek, J., Babula, P., Ferrol, N., Kizek, R., Adam, V., 2013. Metal transporters in plants. In: Gupta, D. K., Corpas, F.J., Palma, J.M. (Eds.), Heavy metal stress in plants. Springer-Verlag, Berlin Heidelber, pp. 19-41.

Zuzarte, M., Gonçalves, M.J., Cavaleiro, C., Dinis, A.M., Canhoto, J.M., Salgueiro, L.R., 2009. Chemical composition and antifungal activity of the essential oils of *Lavandula pedunculata* (Miller) Cav.. Chemistry & Biodiversity 6, 1283-1292.

Zuzarte, M., Dinis, A.M., Cavaleiro, C., Salgueiro, L., Canhoto, J.M., 2010. Trichomes, essential oils and in vitro propagation of *Lavandula pedunculata* (Lamiaceae). Industrial Crops Products 32, 580-587.

2. INTER-POPULATION VARIATION ON THE ACCUMULATION AND TRANSLOCATION OF POTENTIALLY HARMFUL CHEMICAL ELEMENTS IN CISTUS LADANIFER L. FROM BRANCANES, CAVEIRA, CHANÇA, LOUSAL, NEVES CORVO AND SÃO DOMINGOS MINES IN THE PORTUGUESE IBERIAN PYRITE BELT

ABSTRACT

This study aimed to compare the variation on the accumulation and translocation of potentially harmful chemical elements and nutrients (As, Ca, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn) in *Cistus ladanifer* L. belonging to populations growing in different mine areas from the Portuguese Iberian Pyrite Belt (Brancanes, Caveira, Chança, Lousal, Neves Corvo, São Domingos). These mines are abandoned, (except Neves Corvo that is still operating) and have different contamination levels.

Composite samples of soils (*n* = 31), developed on different mine wastes and/or host rock, and *C. ladanifer* plants (roots and shoots) were collected in the mine areas. Soils were characterized for pH, NPK and organic C, by classical methodologies. Soils (total fraction − four acid digestion, and available fraction − extracted with aqueous solution of diluted organic acids, simulating rizosphere conditions) and plants (ashing followed by acid digestion) elemental concentrations, were determined by ICP. Soil-plant transfer and translocation coefficients were calculated. Principal Components Analysis in both ways, the classical method and a second approach with adaptations used mostly in multivariate statistical processes control data, were done in order to compare the plants populations.

Soils had large heterogeneity in their characteristics. Caveira, Lousal, Neves Corvo and São Domingos soils showed the highest total concentrations of As, Cu, Pb and Zn. Independently of the mine, available fractions of elements were low. Intra and inter-population variations in accumulation and translocation of elements were evaluated. Plants were not accumulators of the majority of the analysed elements. Nutrients were mainly translocated from roots to shoots, while trace elements were stored in roots (except in Neves Corvo for As and Pb, and São Domingos for As). Elements concentrations in plant populations from Lousal, Chança and São Domingos did not present much variation. Brancanes soils and plants presented strong differences compared to other areas.

Cistus ladanifer plants are able to survive in mining areas with polymetallic contamination at different elements concentrations in total and available fraction. This species presented variations inter- and intra-populations in accumulation and translocation of chemical elements, however all studied populations, except Brancanes, can belong to the same population cluster.

KEYWORDS Iberian Pyrite Belt • Multivariate statistical process control • Nonlinear Iterative Partial Least Square (NIPALS) • Principal Components Analysis • Portugal

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INTRODUCTION

Over the time, the ore extraction and disposal of mining wastes in an uncontrolled way caused significant environmental problems, namely acid generation, high total concentrations of trace elements in tailings, soils and sediments that can reduce or even inhibit the specific diversity of plant community (Abreu and Magalhães, 2009; Wong, 2003). These problems can also be observed in the mines of the Portuguese Iberian Pyrite Belt (PIPB) (Matos and Martins, 2006a).

Although in the mining areas can coexist many stress factors for the plants as, for instance, high total concentrations of chemical elements in the soil, low pH, low organic matter content, soil water deficit, combined with high radiation and air temperature in summer in mine areas from Mediterranean region, some tailings and soils developed on mine wastes are naturally colonized by spontaneous vegetation. These plants populations usually represent specific ecotypes well adapted to mining conditions constituting communities with small diversity. The development of this tolerant vegetation contributes to the wastes weathering and pedogenesis and the evolution of ecological succession (Abreu and Magalhães, 2009).

Cistus ladanifer L. is one of the species identified in several mining areas and its ecological behaviour has been studied in each contaminated area by several authors (Abreu et al., 2009, 2011; Alvarenga et al., 2004; Batista et al., 2007; Chopin and Alloway, 2007; de la Fuente et al., 2010; Freitas et al., 2004; Lázaro et al., 2006; Murciego et al., 2007; Pratas et al., 2005; Reglero et al., 2008; Santos et al., 2009; 2012). This sbrub is a plastic species that shows biological adaptations, as an efficient system of antioxidant enzymes (Santos et al., 2009) allowing its development in contaminated mine areas like São Domingos mine. However, studies involving an analysis of several *C. ladanifer* populations from different mine areas are scarce (Abreu et al., 2011; Batista et al., 2007; Chopin and Alloway, 2007). Furthermore, the majority of the studies were practically focused on copper, lead and zinc, and sometimes As, while the *C. ladanifer*–soil relationships regarding nutrients are weakly known.

This study aimed to compare the variation on the accumulation and translocation of potentially harmful chemical elements and nutrients (As, Ca, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn) in *Cistus ladanifer* populations growing in different mine areas from the Portuguese Iberian Pyrite Belt (Brancanes, Caveira, Chança, Lousal, Neves Corvo, and São Domingos).

MATERIALS AND METHODS

Site characterization

Six mining areas located in Alentejo region in the Portuguese Iberian Pyrite Belt were studied: Brancanes, Caveira, Chança, Lousal, Neves Corvo and São Domingos mining areas. These mines are nowadays abandoned, except Neves Corvo that is still operating.The location of these mines in PIPB region is shown in Figure 1; Caveira and Lousal mines are located in the NW part (±12 km away from each other); Brancanes and Neves Corvo are situated in the SW region (±3 km away from each

other); while São Domingos and Chança are located in the SE part of the PIPB (±5 km away from each other).

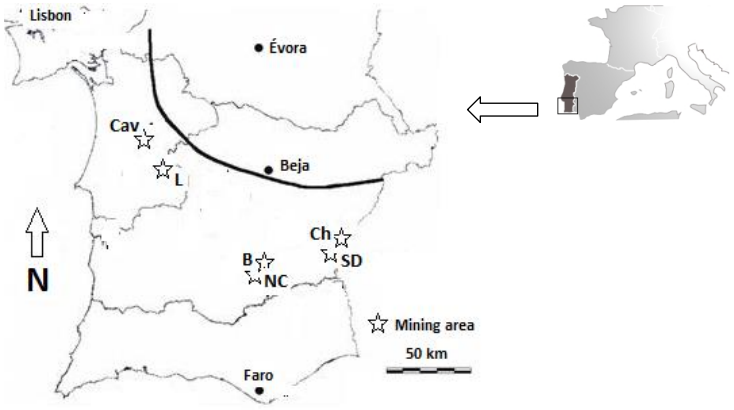

Fig. 1 Location of the studied mining areas in the Portuguese Iberian Pyrite Belt (B: Brancanes; Cav: Caveira; Ch: Chança; L: Lousal; NC: Neves Corvo; SD: São Domingos).

These mines were exploited in different periods of time, and the size of the massive sulfide deposits was also different. According to Matos and Martins (2006a), Neves Corvo present giant deposits (> 200 Tg) whereas Lousal, São Domingos and Caveira were medium deposits (5–50 Tg) and Chança was a minor deposit (< 5 Tg). Brancanes corresponds to a copper vein structure.

During Roman times, massive sulfide deposits and/or *gossan* from Caveira, Lousal, São Domingos and Chança were intensively exploited. The modern exploitation started in the XIXth century in Caveira (1854), São Domingos (1857), Chança (1877) and Lousal (1900), and in the XXth century in Neves Corvo (1987) (Matos and Martins, 2006a,b; Matos et al., 2008; Quental et al., 2002). Brancanes mine was only exploited during the XIXth century being closed by the end of that century, while other mines ceased their activity in the XXth century (Chança: 1930's; São Domingos 1960's; Caveira 1970's; Lousal: 1980's).

In the PIPB context, the Caveira, Lousal, and São Domingos mines generate large negative environmental impact, due to the dimension of the areas affected by tailings and by the acid mine drainage (AMD) (Matos and Martins, 2006a; Pérez-López et al., 2008), while Brancanes and Chança are nowadays areas showing a minimum environmental impact, and may be considered in process of natural attenuation (Abreu et al., 2011; Matos and Martins, 2006a).

The climate in these mining areas is typically Mediterranean characterized by long hot and dry summers, and winters moderately cold and wet. The average annual rainfall lies between 432 and 500 mm (INMG, 1990) and occurs mostly in winter and with an irregular shape.

Field sampling

Several sampling areas (n = 31) with ≈10 m^2 each were selected to include representative soils of each mine where *Cistus ladanifer* L. was growing up. These soils were developed on different mine wastes, mixtures in variable proportions of wastes and host rocks, being considered Spolic Technosol

Toxic (IUSS Working Group WRB, 2007). In some cases, soils are developed on schists and greywackes (Leptosol; IUSS Working Group WRB, 2007) and can be influenced by adjacent tailings and/or AMD. Soils are developed on: schists and greywackes in Brancanes (*n* = 3); schist, colluvium/alluvium materials, slags with different ages and massive and semi-massive sulfide ore in Caveira (*n* = 7); *gossan* materials and crushed pyrite in Chança (*n* = 6); schist in Lousal (*n* = 2); schists and greywackes in Neves Corvo (*n* = 7); and *gossan* materials, modern slags and pyrite ashes in São Domingos (*n* = 6).

In each sampling area, composite samples of soils (≈3 kg of homogenate of, at least, three trial points) were collected in the surrounding of the *C. ladanifer* radicular system (0–20 cm). At the same time and in the same sampling area were also collected roots and shoots (composed of leaves and twigs) of *C. ladanifer* plants. Plant samples represent, as for the soils, composite samples of a homogenate of at least three plants representing a population of *C. ladanifer* growing in each sampling area.

Samples characterization

Soils

The soil samples were air-dried, homogenized and sieved. The soil fraction (< 2 mm) was characterized for (Póvoas and Barral, 1992): pH and electrical conductivity (EC) in water suspension (1:2.5 *m*/*V*); organic carbon (Tinsley method); extractable phosphorous and potassium (Egner-Riehm method) and total nitrogen (Kjeldahl method).

The total concentrations of As, Ca, Cu, Fe, Pb, Mg, Mn, Ni, K and Zn (fraction < 2 mm) in soils were determined in an international certified laboratory using ICP and INAA, after acid digestion with perchloric acid + nitric acid + hydrochloric acid + hydrofluoric acid (Actlabs ISO/IEC 17025; Activation Laboratories, 2013a).

The same chemical elements were analysed in a soil extractable aqueous solution that simulated rizosphere conditions (RHIZO solution; Feng et al. 2005) which contain acetic acid + lactic acid + citric acid + malic acid + formic acid at 10 mmol/L. These extractable soil solutions were analysed by ICP-MS and ICP-OES in the same certified laboratory (Activation Laboratories, 2013b).

Plants

Roots and shoots samples were washed with tap water and then with distilled water. The roots were still sonicated in distilled water for 30 minutes. Plant samples were dried (40 °C) homogenised and finely ground. The same chemical elements than in soils were analysed by ICP-MS, after ashing (475 °C) and nitric acid digestion (Activation Laboratories, 2013c).

Data analysis

Descriptive statistics was performed in the four sets of data – total concentrations of chemical elements in the soils, available fraction of soils, roots and aerial parts of the *C. ladanifer* from all

mining areas. Measures of central tendency and dispersion were obtained. Principal Components Analysis (PCA) in two ways – the classical method and a second approach with adaptations used mostly in multivariate statistical process control data – were done assuming that the populations of variables are multivariate normally distributed. To assess multivariate normality Probability-Probability chart was produced to check the tendency of all variables together to follow the normal theoretical curve. In the second approach instead of rotations of the axis in classical PCA, where is tried to maximize the highly correlated data and minimize the lowest correlations of data, it is proposed in this approach the change of the origin of data to the mean. Sometimes it is necessary to change the origin of data to the mean more than once to have the best fit. The transformed variables are the ratio between, the difference of the original variable and the mean, in numerator, and the standard deviation of the original variable, in denominator. This second method of application of PCA was performed in this study using the Nonlinear Iterative Partial Least Square (NIPALS) (an iterative procedure) algorithm with 50 iterations maximum number and convergence criteria of 0.0001. The iterative method NIPALS used to build the principal components model (find the eigenvectors) for the representation of multivariate variables X (Risvic, 2007). The number of components was fitted by cross-validation. Another important difference between the classical PCA and the second approach PCA is the significance and limit of the principal components. Classical PCA significance of principal components determines the number of principal components until all the data are explained. This way, the number of principal components corresponds to the number of variables. In the second method, the limit and the significance of the principal components is based on the method of cross-validation. The cross-validation method was V-fold validation. In V-fold cross validation, the data are divided into *v* segments, *v*-1 of which is used to build up the model and the rest is used for testing. This process is repeated for all possible permutations of the training and testing segments and the overall Q^2 and Q^2V are calculated for the newly added principal component using the test samples. Given the estimated values of Q^2 and Q^2V, it is possible to determine, whether a principal component is significant.

The reason for the choice of both methods was that the soil and plant data were obtained in different mining areas with different extraction of ore volumes, which could impact differently the environment. As was already referred, these mining areas had different periods of mining and one of them is still in operation.

Classical PCA is considered to organize variables in clusters. It is also important to obtain the usual interpretations and tests what can be obtained with the second approach. Adding this PCA control process it is expected to extract more information from the data and at the same time, perform a quality control of the data. This method is usually obtained in the industry to control the quality of data produced. Apart from the called “quality control” that it is considered necessary because of the diversity of mining areas with different ore deposits extracted in different time periods, it is possible to have information from the predictors and from the residuals. Predictors correspond to pre-processing the data and with it, predict future observations, and in the present case try to eliminate over representation of some variables, merely on the basis of, for instance, the range of data. Variables often have substantially different ranges (*i.e.*, different variances). This may have to do with the units of measurements or simply the nature of the variables themselves. However, the numeric range of a

variable may not be a good indication of the importance of that variable. Residuals, on the other hand, correspond to the properties that are not captured by the principal components but that can contain important information such as outliers. These outliers are observed in the Hotelling T2 diagram or D-to-Model diagram (Distance to the model) where moderate outliers are identified. The degree of freedom is given by the difference between the variables number and the principal components. At the end, predictions for the PC model plus residual variance equal the preprocessed data (scaled) (Hill and Lewicki, 2007).

The four principal components (PC) are represented by the lines and from the bottom line until the first, respectively PC1 to PC4. The groups of variables that are correlated with the respective PCs are represented in relation to the respective lines and the contributions are cumulative in the respective diagrams. Therefore, the biggest contribution is given by PC1 and the other are added to this one.

For statistical purposes, the results below the detection limit were assumed as half of the detection limit while for the results over range was considered the limit value range. Quality control of the analysis was made by analytical replicate samples, use of certified standard solutions and laboratory standards at the Activation Laboratories.

Soil-plant transfer and translocation coefficients were calculated. Soil-plant transfer coefficient (TransferC = [total shoots element]/[total soil element]), also named biological absorption coefficient or biological accumulation coefficient, indicates if the plants can be considered as accumulator (TransferC > 1) or non-accumulating of a specific element. The translocation capacity of an element, from roots to shoots, can be evaluated by translocation coefficient (TranslC = [total shoots element]/[total roots element]).

RESULTS AND DISCUSSION

Soil characteristics

The characteristics of the soils collected in each mining area had great variability, depending on the complex mixture of mine wastes or mine wastes plus host rocks (Table 1). The pH of the soils can be considered moderately acid or acid, with values below 4, in areas that uses to receive the influence of acid mine drainage or where soils have been developed on mine wastes containing sulfides, as is the case of the majority of the PIPB mines. The soils of Brancanes had the pH in the range 5.4 – 6.0 because the collected samples were developed on host rocks (Table 1).

The high values of the electrical conductivity of the soils from Lousal (> 1.3 mS/cm) is a consequence of the influence of waste dump leachates leading to salt efflorescence formation on the soil surface in the summer season. The soils were collected near the bottom of a waste pile, draining directly to the place.

Concentrations of extractable K in soils from all mine areas were in the range considered medium-high or very high (INIA – LQARS, 2000), depending on mine area. In general, soil fertility in Brancanes and Neves Corvo was slightly better, compared to the soils of other mine areas, because the concentrations of organic C, total N and extractable P in soils of those sampling areas can be considered as medium to high or very high (INIA – LQARS, 2000) (Table 1).

Comparing the concentrations of the studied chemical elements in the soils of the different mine areas, soils from Brancanes presented the lowest values for the total concentrations of the elements (except for manganese; Table 2). The same tendency was not verified for the soil available fraction where the concentrations of Ca, Fe, Mg, Mn and K (Table 3) in the extractable solutions were among the highest values when compared to the other soils. For the six mine areas, the total concentrations of As in the soils exceeded the maximum allowed values (MAV) according to the Canadian legislation (12 mg As/kg; CCME, 2007).Total concentrations of Cu and Pb in the majority of the soils from all the mine areas, with Brancanes exception, were above the MAV for agriculture use and following the Canadian Soil Quality Guidelines (63 mg Cu/kg and 70 mg Pb/kg; CCME, 2007). Unlike As, Cu and Pb the total concentration of Zn were below the MAV (200 mg Zn/kg; CCME, 2007) in São Domingos soils and some soil samples from Neves Corvo. Nickel total concentrations in all the mine soils were relatively low and within the range of the medium values calculated by Kabata-Pendias (2011) on the World Scale for Different Soil Groups and even below the maximum allowed values according to CCME (2007) for any soil use (50 mg Ni/kg). Soils from Chança and São Domingos presented lower total concentrations of manganese than the soils of the other mine areas.

The total concentrations of the trace elements in the mine soils were high (Table 2), but the concentrations of the same elements in the soil available fraction were, in general, low in all mine areas (Table 3). Although elements concentrations in available fraction differ according to the used extraction solution, low concentrations of arsenic, copper, iron, manganese, lead and zinc in the soil available fractions were also observed in Caveira and São Domingos (Abreu et al., 2012a,b; Batista et al., 2009; Santos et al., 2012) as well as in some soil available fractions from Neves Corvo and Brancanes (Batista et al., 2007). In Caveira and São Domingos soils, the low availability of As, Cu, Pb, Mg and Zn was explained by the association of these elements mainly to the residual fraction (accounted to solid phases with low solubility) as well as Fe-oxide fractions and, in some soils from São Domingos, by the presence of solid phases, in the residual fraction, containing As, Fe and Pb (Abreu et al., 2012b; Santos et al., 2012).

The As, Cu, Fe, Pb, Mg, Ni and Zn total concentrations in the soils of the studied mines are in the same range than those reported, by several authors, for soils collected from mines of the Iberian Pyrite Belt (Abreu et al., 2012a,b; Álvarez-Valero et al., 2008; Pérez-López et al., 2008) or for soils from mines where *C. ladanifer* grows (Table 4).

Table 1 Characteristics of the soils from studied mining areas (minimum – maximum; geometric mean).

	Brancanes (*n* = 3)	**Caveira (*n* = 7)**	**Chança (*n* = 6)**	**Lousal (*n* = 2)**	**Neves Corvo (*n* = 7)**	**São Domingos (*n* = 9)**
pH (H_2O)	5.4 – 6.0 5.7	3.9 – 6.2 4.7	3.7 – 5.7 4.7	4.0 – 4.5 4.2	3.5 – 5.9 4.9	3.5 – 4.5 4.0
EC (µS/cm)	nd	122 – 286 179	nd	2540 – 1343 1849	nd	130 – 603 267
Organic C (g/kg)	39.8 – 75.4 45.0	13.0 – 64.3 33.3	13.8 – 33.8 23.0	3.8 – 6.5 4.9	25.2 – 81.9 47.9	5.9 – 22.4 15.1
Total N (g/kg)	2.2 – 5.9 3.2	0.02 – 2.7 0.5	0.7 – 1.3 1.0	0.6 – 0.7 0.6	2.0 – 5.3 3.5	0.6 – 1.9 1.2
Extractable K (mg/kg)	465 – 1436 557	58.1 – 324 129	49.3 – 90.8 65.8	41.5 – 43.2 42.3	27.4 – 444 223.6	45.6 – 143 71.7
Extractable P (mg/kg)	62.6 – 367 81.7	<0.9 – 74.3 2.1	2.6 – 11.7 4.3	0.9 – 1.8 1.3	16.1 – 75.9 36.5	<0.9 – 2.7 <0.9

Nd: not determined; EC: electrical conductivity

Table 2 Total concentrations of chemical elements in soils from studied mining areas (minimum – maximum; geometric mean).

	Brancanes (*n* = 3)	**Caveira (*n* = 7)**	**Chança (*n* = 6)**	**Lousal (*n* = 2)**	**Neves Corvo (*n* = 7)**	**São Domingos (*n* = 6)**
			(*mg/kg dry weight)*			
As	28 – 45 33	133 – 1.3x10^3 525	63 – 151 102	198 – 426 290	18 – 297 59	711 – 1.8x10^3 1.4 x10^3
Cu	38 – 60 45	196 – 2.8x10^3 522	30 – 504 153	232 – 245 238	179 – 6.1x10^3 1.2 x10^3	203 – 379 287
Mn	495 – 1.5x10^3 918	181 – 2.4x10^3 672	61 – 797 236	1.1x10^3 – 2.4x10^3 1.6x10^3	403 – 3.2x10^3 1.1 x10^3	130 – 575 259
Ni	32 – 40 36	14 – 36 22	6 – 49 15	35 – 50 42	12 – 42 26	16 – 48 30
Pb	15 – 38 22	932 – 48x10^3 5.4 x10^3	48 – 119 84	432 – 721 558	19 – 175 51	666 – 5.8x10^3 1.9x10^3
Zn	32 – 55 39	193 – 785 381	32 – 121 62	350 – 497 417	68 – 607 215	113 – 186 137
			(*g/kg dry weight)*			
Ca	0.2 – 0.6 0.3	0.4 – 2.8 1.3	0.6 – 8.4 1.2	1.6 – 3.3 2.3	1.5 – 6.8 3.0	0.4 – 1.2 0.7
Fe	2.9 – 3.4 3.1	38.8 – 114 58.4	57.5 – 123 86.7	64.4 – 73.9 6.9	23.8 – 47.6 38	51.9 – 136 83.1
K	1.6 – 2.4 2.0	20.3 – 28.9 23.9	7.1 – 29.5 15.4	25.4 – 29.4 27.3	9.5 – 26.4 16.3	15.1 – 20.3 17.7
Mg	0.2 – 0.5 0.3	3.3 – 6.4 4.5	1.5 – 35.9 4.7	8.3 – 11.7 9.9	3.8 – 7.7 5,3	1.4 – 5.9 3.5

Table 3 Concentrations of chemical elements in the available fraction of the soils from the studied mining areas (minimum – maximum; geometric mean).

	Brancanes (*n* = 3)	**Caveira (*n* = 7)**	**Chança (*n* = 6)**	**Lousal (*n* = 2)**	**Neves Corvo (*n* = 7)**	**São Domingos (*n* = 6)**
			(*mg/kg dry weight)*			
As	< 0.1	0.1 – 8.5 0.5	< 0.1	0.3 – 0.4 0.3	< 0.1	0.2 – 1.9 0.5
Ca	431 – 1.6x10^3 952	96 –> 200 176*	24.1 – 408 87.7	> 200	467 – 3.5x10^3 1.0 x10^3	57.0 – > 200 118*
Cu	0.5 – 2.6 1.3	1.2 – 286 5.4	0.4 – 13.4 1.6	6.6 – 8.4 7.4	7.0 – 430 43.3	3.3 – 10.0 5.6
Fe	110 – 239 177	91 – > 100 98.6*	20.9 – 162 47.8	59.5 – > 100 77.1	179 – 540 342	29.5 – > 100 81.6*
K	560 – 1.6x10^3 825	36.6 – 116 61.3	9.2 – 72.8 35.0	26.3 – 35.5 30.6	12.0 – 499 148	28.5 – 81.9 39.3
Mg	150 – 418 220	16.9 – 187 80.1	16.2 – 171 60.6	165 – > 200 182*	119 – 210 150	21.5 – 86.9 48.7
Mn	79.8 – 370 184	7.1 – 142 47.3	1.0 – 42.5 7.2	67.9 – 256 132	119 – 460 217	4.0 – 46.8 11.2
Ni	<0.1	0.2 – 1.2 0.3	0.1 – 0.4 0.2	0.6 – 1.3 0.9	<0.1	0.1 – 0.4 0.3
Pb	< 0.02	1.1 – 215 22.6	0.02 – 0.1 0.1	0.5 – 0.8 0.6	< 0.02	0.2 – 40.4 1.0
Zn	0.3 – 4.1 2.5	4.3 – 80.8 12.1	0.9 – 1.4 1.1	32.5 – 45.0 38.2	2.7 – 160 19.0	3.7 – 7.4 5.2

*Estimated geometric mean considering the over range value.

Table 4 Concentrationsof some chemical elements (mg/kg) reported by several authors for *Cistus ladanifer* plants (roots and shoots) growing in mining areas and respective soils where plants were collected.

	As	Cu	Fe	Mn	Ni	Pb	Zn
Total in soils	45 – 565[(1)] 137 – 297[(2)] 235 – 6.3x10^3[(4)] 3 – 6.3x10^3[(5a)] 2 – 15.2x10^3[(5b)] 842 ± 1.3x10^3[(6)] 444[(8)] 11.1 − 651[(9)] 2.6x10^3 ± 610[(11)]	226 – 1.8x10^3[(1)] 179 – 6.1x10^3[(3a)] 28 – 62[(3b)] 32 – 361[(3c)] 368 – 1.4x10^3[(4)] 4 – 690[(5a)] 20 – 3.1x10^3[(5b)] 226 ± 190[(6)] 15 – 255[(7)] 443.7[(8)] 15.5 – 78.2[(9)] 66.4 ± 6.1[(10)] 226.0 ± 14.2[(11)]	47.4x10^3 – 112x10^3[(1)] 23.8x10^3 – 47.6x10^3[(3a)] 28.5x10^3 – 34.4x10^3[(3b)] 18.5x10^3 – 39.8x10^3[(3c)] 65.8x10^3 – 165x10^3[(4)] 73.1 x10^3 ± 70.8 x10^3[(6)]	252 – 1.2x10^3[(1)] 403 − 3.2x10^3[(3a)] 495 – 1.5x10^3[(3b)] 1.2x10^3 – 4.1x10^3[(3c)] 217 – 542[(4)] 284 ± 420[(6)]	15.1 ±11.8[(6)] 46.1[(8)]	301 – 3.5x10^3[(1)] 23 – 175[(3a)] 12 – 38[(3b)] 38 – 139[(3c)] 603 – 14.5x10^3[(4)] 14 – 24.8x10^3[(5a)] 18 – 6.4x10^3[(5b)] 889 ± 1.1x10^3[(6)] 9.9 – 34.7[(7)] 3.6x10^3[(8)] 7.8 ± 1.4[(10)] 7.3x10^3 ± 2.0 x10^3[(11)]	140 – 945[(1)] 78 – 607[(3a)] 32 – 59[(3b)] 28 – 286[(3c)] 161 – 697[(4)] 16 – 420[(5a)] 45 – 870[(5b)] 126 ± 71.4[(6)] 33.4 – 118[(7)] 230.8[(8)] 31.2 ± 1.9[(10)] 43.3 ± 11.9[(11)]
Roots	0.5 – 6.7[(2)] 12.0 ± 3.7[(11)]	5.8 – 80.6[(1)] 9.1 – 176[(3a)] 2.8 – 9.1[(3b)] 4.5 – 8.7[(3c)] 9.4 ± 6.3[(11)]	382.5 – 1.1x10^3[(3a)] 216 – 1.7 x10^3 [(3b)] 289 – 2.8 x10^3[(3c)]	85 – 357[(1)] 64 – 5.6 x10^3[(3a)] 231 – 581[(3b)] 253 – 590[(3c)]	---	2.7 – 20.1[(1)] 0.6 – 3.5[(3a)] 0.2–1.6[(3b)] 0.7 – 7[(3c)] 53.9 ± 18.9[(11)]	18.2 – 92.5[(1)] 13.8 – 70.7[(3a)] 3 – 13[(3b)] 11.6 – 29.8[(3c)] 41.2 ± 12.3[(11)]
Aerial part/Leaves/ Plants tops	<10[(1)] 2.5 – 42.0[(2)] 4.9 – 16.0[(4)] 0.3 − 30[(5a)] 1 – 22[(5b)] 9.5 ±7.8[(6)] 2.1[(8)] 0.48 – 2.77[(9)] 1.7 ± 0.2[(11)]	8.3 – 29.7[(1)] 64.4 – 591.5[(3a)] 7.1 – 10.9[(3b)] 22.6 – 98[(3c)] 4.1 – 20.2[(4)] 7 – 21[(5a)] 7 – 42[(5b)] 26.3 ± 12.3[(6)] 30.3 – 330[(7)] 13.4[(8)] 16 – 460[(9)] 12 ± 0.5[(10)] 8.2 ± 0.6[(11)]	645 – 3.8x10^3[(1)] 579 – 4.6x10^3[(3a)] 351 – 969[(3b)] 313.2 – 1.8x10^3[(3c)] 63.8 – 716[(4)] 724 ± 433[(6)]	117 – 1.3x10^3[(1)] 212.9 – 1.2x10^3[(3a)] 558 – 1.2x10^3[(3b)] 445 – 1.2x10^3[(3c)] 84.6 – 2.0x10^3[(4)] 664 ± 593[(6)]	3.51±2.03[(6)] 4.6[(8)]	< 10 – 26.0[(1)] 2.4 – 24.1[(3a)] 0.6 – 0.8[(3b)] 1.1 – 4.5[(3c)] 3.0 – 276[(4)] 2 – 237[(5a)] 7 – 102[(5b)] 10.6 ±12.6[(6)] 3.3 – 16.3[(7)] 11.0[(8)] 13.6 ± 2.7[(10)] 55.8 ± 6.3[(11)]	99 – 583[(1)] 54.4 – 177[(3a)] 12 – 19.8[(3b)] 60.4 – 154.5[(3c)] 56.4 – 391[(4)] 53 – 280[(5a)] 70 – 285[(5b)] 113 ±80.9[(6)] 26.1 – 101[(7)] 170.1[(8)] 73 – 729[(9)] 98.5 ± 1.0[(10)] 147.9 ± 10.1[(11)]

[(1)]Alvarenga et al. (2004), soils, roots and leaves from Aljustrel mine; [(2)]Batista et al. (2004), soils, roots and leaves from Neves Corvo mine; [(3)]Batista et al. (2007), soils, roots and leaves from Neves Corvo mine[(a)], Brancanes mine[(b)], old mines in the NW area[(c)]; [(4)]Batista et al. (2009), soils and leaves (young and mature) from São Domingos mine; [(5)]Chopin and Alloway (2007), soils and leaves from Tharsis[(a)] and Rio Tinto mines[(b)]; [(6)]de la Fuente et al. (2010), soils and aerial part from Rio Tinto mine; [(7)]Farago et al. (1992), soils and plants tops from Neves Corvo mine; [(8)]Freitas et al. (2004), soils and leaves from São Domingos mine; [(9)]Pratas et al. (2005), soils and leaves from Sarzedas mine; [(10)]Reglero et al. (2008), soils and leaves from Horcajo mine; [(11)]Santos et al. (2012), soils, roots and leaves from São Domingos mine.

Accumulation and translocation of chemical elements in plants

Concentrations of the chemical elements in roots and shoots of *C. ladanifer* from the different mining areas showed different trends and variability (Figs. 2 and 3). Some populations showed high concentrations of some elements in their shoots, as is the case of As in plants from Neves Corvo and São Domingos and lead in plants from Caveira. In fact, the accumulation capacity for elements in *C. ladanifer* shoots depends on element and population (Kidd et al., 2004; Lázaro et al., 2006; Santos et al., 2012, 2013). Considering all the populations as a cluster, the elements concentrations in shoots and roots were not correlated to the concentrations of the same elements in soils (total and available fraction), except for Pb. For the different mine areas, the existence or the absence of this type of correlation vary with soils, plants and chemical elements and, usually, did not show a regular pattern. Some authors also reported both behaviours for this species populations growing in São Domingos mine (Batista et al., 2009; Santos et al., 2012).

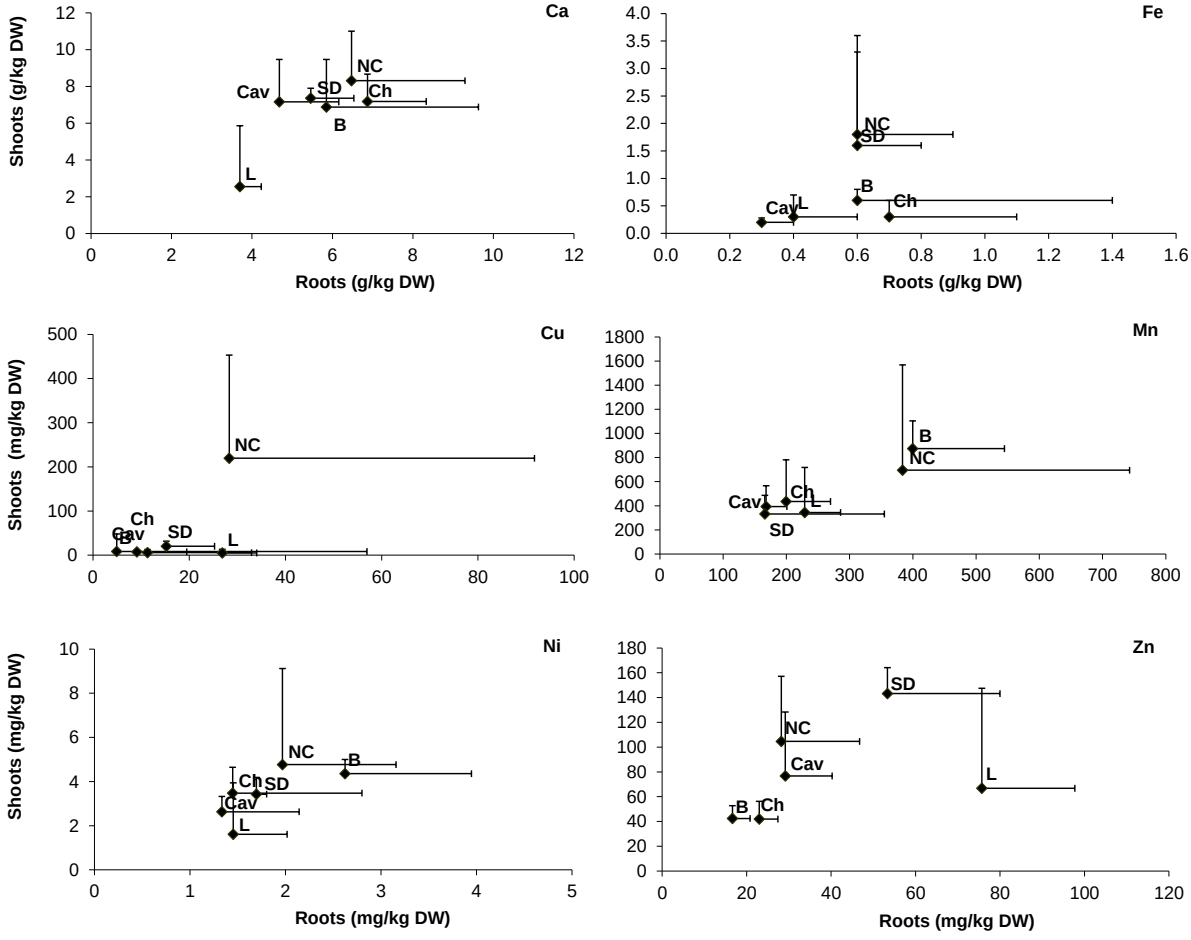

Fig. 2 Concentrations of nutrients in roots and shoots of *Cistus ladanifer* collected in the studied mining areas (geometric mean ± SD) (B: Brancanes; Cav: Caveira; Ch: Chança; L: Lousal; NC: Neves Corvo; SD: São Domingos).

The different concentrations of chemical elements in the shoots are the result of several plant processes, like uptake, accumulation in roots, translocation from roots to shoots and tolerance

capacity rather than soil elements concentrations in the soil available fraction. In fact, some of the studied plants showed higher concentrations of some elements in shoots than in the soil available fraction. Although Lousal population had in shoots and roots similar elements concentrations compared to the plants from the other studied mines (except for Ca and Zn; Figs. 2 and 3), this fact may not reflect the real behaviour of the plant population from Lousal because only two sampling areas were considered for this mine area. Calcium concentrations in both plant parts varied in the same range (3−12 g/kg; Fig. 2) however the plants, except those from Lousal, presented Ca concentrations (average values) around 6 and 7 g/kg for roots and shoots, respectively. The concentrations of this element in *C. ladanifer* shoots collected in the IPB mining areas (Aljustrel: 5.0 − 8.9 g/kg; Alvarenga et al., 2004; Rio Tinto: 5.6 ± 3.4 g/kg; de la Fuente et al., 2010) were in same range. Lousal plants reached the lowest calcium concentrations (g/kg − roots: 3.7; shoots: 2.6).

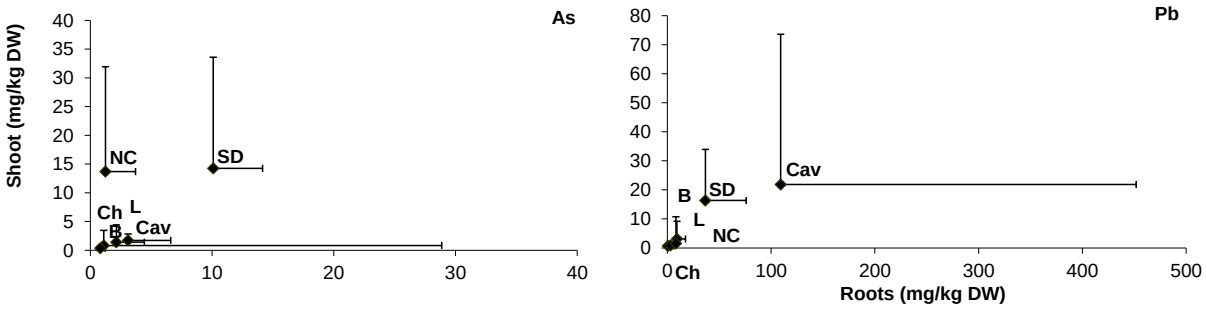

Fig. 3 Concentrations of trace elements in roots and shoots of *Cistus ladanifer* collected in the studied mining areas (geometric mean ± SD). (B: Brancanes; Cav: Caveira; Ch: Chança; L: Lousal; NC: Neves Corvo; SD: São Domingos).

Neves Corvo and São Domigos plants reached the highest concentrations of As in shoots (mg/kg − NC: 13.7; SD: 14.3; Fig. 3) and iron (mg/kg − NC: 1.8 x 10^3; SD: 1.6 x 10^3; Fig. 2). However, the arsenic concentrations in their roots is quite different (mg/kg – NC: 1.2; SD: 10.1) while for Fe concentrations are similar (0.6 x 10^3 mg/kg). Most of the plants from these two mine areas showed arsenic concentrations above the phytotoxic limit reported by Kabata-Pendias (2011; 5 – 20 mg As/kg; > 1x10^3 mg Fe/kg), but visual signs of toxicity were not observed. The absence of As toxicity in *C. ladanifer* can be related to the accumulation of this elements in organic forms within the plant (Batista et al., 2004) and the existence of an efficient detoxification process through of the activity of some antioxidative enzymes (Santos et al., 2009). Furthermore, the increase of As concentration with the leaves development from mature to senescent stage in *C. ladanifer* plants growing on soils developed on *gossan* materials from São Domingos mine can be considered as a detoxification process to assist arsenic removal (Santos et al., 2013). Regarding As in plants in the other mine areas, two clusters can be formed: Brancanes and Chança with As concentrations in shoots and roots lower than 2 mg/kg, and Caveira and Lousal with concentrations varying between 1 and 9.5 mg/kg depending on plant part and sampling area. The Fe concentration in roots and shoots in plants populations from Brancanes, Caveira, Chança and Lousal showed great variability varying between 0.1 and 0.8 g/kg.

The Pb concentration in *C. ladanifer* showed a great variation intra- and inter-populations of the mining areas, attaining the plants from Caveira the highest concentrations both in shoots (21.8 mg Pb/kg) and roots (109.2 mg Pb/kg). Plants from Caveira populations showed a wide range of Pb concentrations but most of the values were lower than 24.4 and 244.2 mg Pb/kg for shoots and roots, respectively. One of the *C. ladanifer* sample from Caveira had Pb concentrations in shoots (153 mg/kg) that exceed the range considered phytotoxic by Kabata-Pendias (2011; 30−300 mg Pb/kg). These plants grew in soils with the highest Pb total concentration (48 x 10^3 mg/kg) but not the high Pb concentration in the soil available fraction. Considering sample as an outlier, and comparing Pb concentrations in plants populations from Caveira and São Domingos, they are in the same range. In Brancanes, Chança, Lousal and Neves Corvo, the Pb concentrations in plant populations were low and in the same range, not exceeding 9.6 and 24.3 mg Pb/kg in shoot and roots, respectively.

Copper concentrations in plants (shoots and roots) from Neves Corvo were clearly different from those in plants collected in the other mine areas (mg/kg − roots: 28.3; shoots: 219.5 mg; Fig. 2). São Domingos populations presented higher Cu concentrations in both plant parts (mg/kg − roots: 15.2; shoots: 20.2) than plant populations from Brancanes, Caveira and Chança. These plants had similar mean concentrations of Cu both in roots and shoots, showing Caveira and Chança a great variability in their Cu concentrations (mg/kg – Cav: roots: 4.4−28.8; shoots: 4.8−14.3; Ch: roots: 3.1−58.1; shoots: 2.7−21.4). The majority of the plants collected in the studied mine areas presented Cu concentrations in shoots within the range considered normal or sufficient (5−30 mg Cu/kg; Kabata-Pendias, 2011).

Nickel was considered as a nutrient because this trace element has a possible essential role in plant metabolism (Srivastava and Gupta 1996), however high Ni concentrations have toxic effects. In all the studied populations, the concentration of this element is below the range considered toxic (10−100 mg Ni/kg) and within the normal range (0.1−5 mg Ni/kg) for plants (Kabata-Pendias 2011). Brancanes and Neves Corvo populations showed the greatest variability for Ni concentration, especially, for roots (Fig. 2). Regarding Mn, plant populations from the six studied mine areas behave similarly to nickel. However, most of the plants had Mn concentrations in shoots within or even above the phytotoxic values (400–1000 mg Mn/kg; Kabata-Pendias, 2011) (Fig. 2).

In general, independently of the population, Zn concentrations in shoots (Fig. 2) were considered normal or sufficient (27−150 mg/kg; Kabata-Pendias 2011). Populations from Lousal and São Domingos presented higher concentration of Zn in the roots than the populations growing in the other mine areas.

Cistus ladanifer shoots and roots collected in different mine areas showed a wide range of concentrations for the same chemical elements (Table 4) being, in some cases, different from the values obtained in present study and for the same elements (Figs. 2 and 3).The differences in the storage of some elements suggest different processes for accumulation and/or transport mechanisms within plants (Zhang et al., 2002). The differences can be induced by seasonal and temporal variations in plants and soils (*e.g.* elements concentrations in the soil available fraction, meteorological conditions, water regime), as well as the analysed plant part collected (*e.g.* leaves (young and

mature), shoots (leaves and twigs)) (Alvarenga et al., 2004; Kabata-Pendias, 2011; Murciego et al., 2007; Santos et al., 2012).

The calculated values of the soil–plant transfer coefficient (TransfC) indicated that the populations from the studied mining areas are non-accumulators of As, Cu, Fe, Pb and Ni (TransfC < 1), as was already reported for São Domingos plants growing in soils developed on *gossan* (As, Cu and Pb; Santos et al., 2012) as well as for some *C. ladanifer* plants collected in Aljustrel (PIPB) (Cu and Pb; Alvarenga et al., 2004), and in Tharsis and Rio Tinto mine areas (IPB) (for As, Cu and Pb; Chopin and Alloway, 2007).

Variations inter- and/or intra-population in the accumulation behaviour of Ca, Mn and Zn were observed for the plants collected within the six mine areas. The same variations were already reported for the same species from IPB mines by several authors (Abreu et al., 2011; Alvarenga et al., 2004; Chopin and Alloway, 2007; Santos et al., 2012). In general, this species was Ca accumulator (except one sample from Lousal and other from Neves Corvo) while for the Mn plants presented both behaviours. Plants from São Domingos and Brancanes were Zn accumulators, but the same behaviour was not observed for populations from the other four areas, except one sample from Neves Corvo and two samples from Chança. The same plants species growing in Rio Tinto mine (IPB) were manganese accumulators but excluders of As, Cu, Fe, Pb, Ni and Zn (de la Fuente et al., 2010). Chopin and Alloway (2007) also reported both accumulation behaviours of Zn in *C. ladanifer* populations from Tharsis and Rio Tinto mine areas, while Santos et al. (2012) observed that São Domingos plants were only Zn accumulator plants. According to the concentrations referred in the literature in order to consider a plant as hyperaccumulator of a specific element (Kabata-Pendias, 2011), none *C. ladanifer* population is considered a hipperaccumulator of the Ca, Mn and Zn.

The calculated soil to plant coefficient transfer Caveira, Chança, Lousal and São Domingos mine areas followed the order: Ca > Mn > Zn > Ni > Cu > As, Fe, Pb. This order agrees with that reported by Kabata Pendias (2011) for the majority of the plant species, except for Mn and Ni. Brancanes and Neves Corvo populations presented the same tendency, for the same coefficient except for the Fe and Ni in Brancanes and As in Neves Corvo. The soil to plant coefficient transfer calculated by de la Fuente et al. (2010) for several elements and species, including *C. ladanifer*, from Rio Tinto mine allowed to conclude that the general pattern of elements accumulation was related to the essential requirements for any plant species, corresponding to macronutrients (Ca, Mg) > micronutrients (Na > Fe, Mn > Cu, Zn) > toxic elements (As, Pb).

As for the accumulation behaviour, a variation inter- and intra-population in the elements translocation from roots to shoots was observed (TranslC: 0.1−37.9 depending on population and element). The same behaviour was also indicated by other author for the same species (Abreu et al., 2011; Alvarenga et al., 2004; Santos et al., 2012). Nutrients were mainly translocated from roots to shoots, independently of population, due to their important metabolic functions (TranslC: 1.0–30.6, depending on population and element). The majority of the plants from Chança, Caveira and Lousal showed a low translocation of Cu and Fe from roots to shoots, however the concentrations of these elements in their shoots are considered normal and sufficient. Copper retention in the roots of the

plants growing in soils with high concentrations of this element can be a tolerance mechanism that guarantees adequate levels of Cu in photosynthetic parts of the plant.

Although Neves Corvo soils had low concentrations of As and Pb in total and available fractions (Tables 2 and 3), plants from this area translocated these non-essential elements from shoots to roots (Translocation coefficients of 6.9−37.9 and 1−33.5 for As and Pb, respectively). The high As translocation factor was also observed in São Domingos population, despite the total concentration of As in soils of this area was higher (Table 2). However, an opposite behaviour of As translocation was observed by Santos et al. (2012) for the same species and same mine area but on soils with highest As concentrations than those measured in the present studied soils.

Multivariate analysis

The Principal Components Analysis (PCA) assumes that the studied variables tend to multivariate normality. The Probability-Probability chart (P-P) showed that the sets of variables tend to the theoretical curve (normal) as a whole. However, when those are separated the same tendency does not occur. The diagrams were produced using the elements concentrations in the soils (total and available fractions), and in the aerial parts (shoots) and in the roots of the plants. The best tendency to normality was observed in the P-P diagrams of the soils. Even so, certain elements (Ca, Fe and Ni) seem to deviate more than others from that tendency. However, the deviation is more evident in the P-P diagram for elements concentrations in roots (Fig. 4).

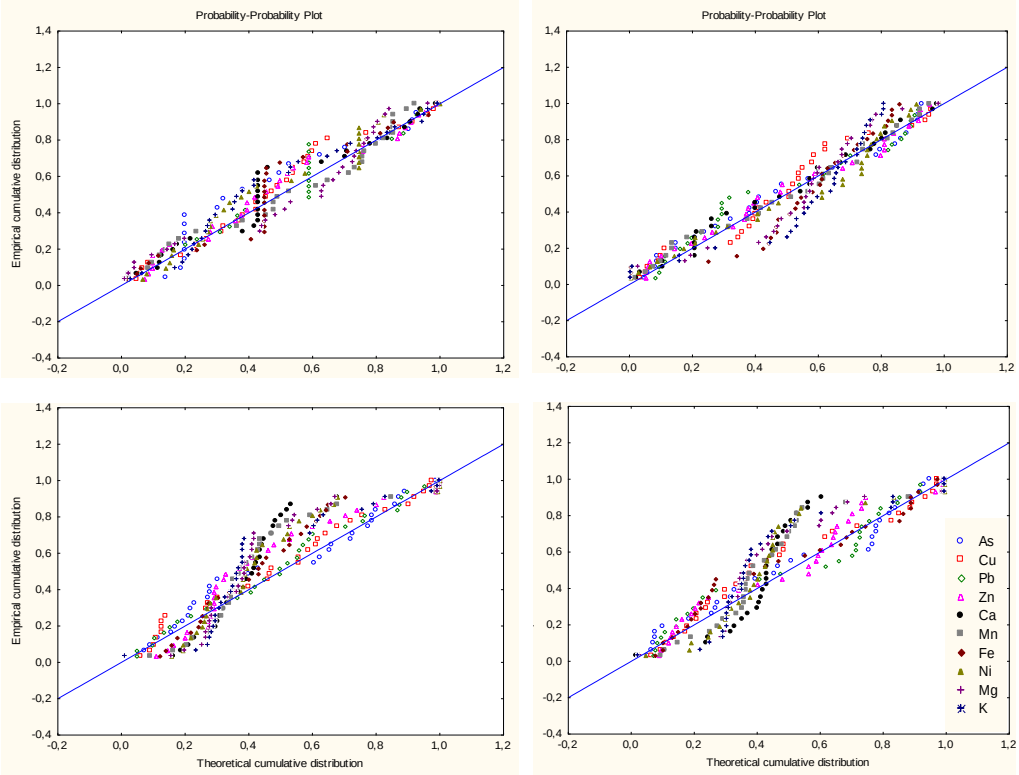

Fig. 4 Probability-Probability plot (P-P) representing empirical cumulative distribution *Vs* theoretical cumulative distribution of the elements concentrations testing possible multivariate normality.

Figure 5 shows that the same groups of elements were obtained in PCA and in PCA-NIPALS, representing the PC1-PC2 axis the available fractions of the elements in soils. PCA-NIPALS provides more information about PCs variables and individuals, so the observations will focus more in this analysis. Table 5 represents the summary of the calculation of the four principal components determined by the prediction model (Q^2cumul) with the respective cumulative variance explanation R^2X for all sample type analysed. The elements concentrations in soils available fraction, total concentrations of the elements in soils, and elements concentrations in the aerial parts and in the roots of the plants have an explained variance of 81.95 %, 70%, 96.87 % and 98 %, respectively.

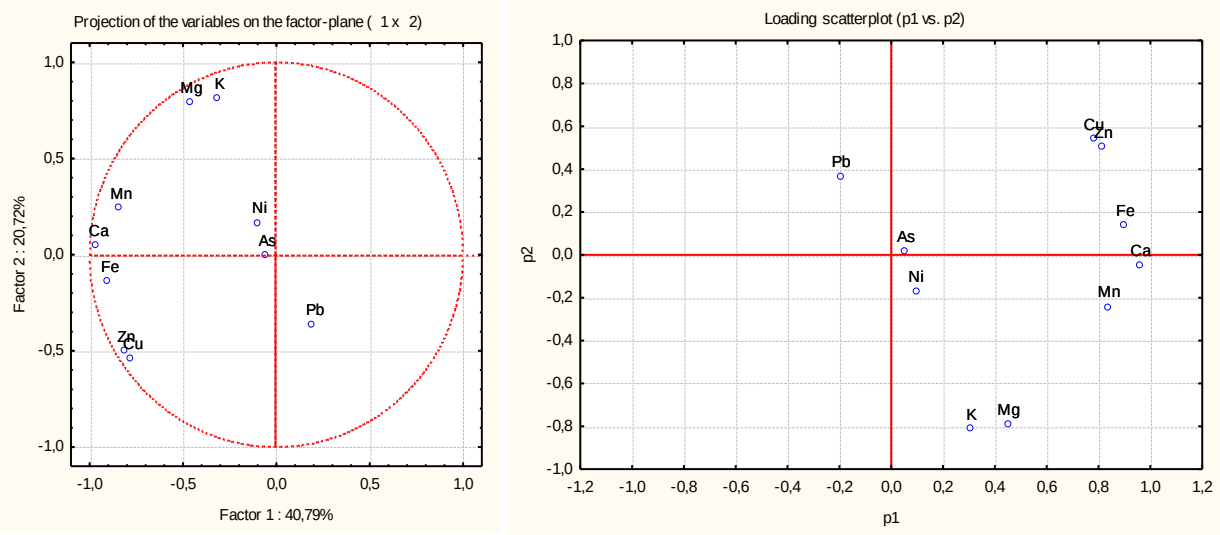

Fig. 5 PC1-PC2 plots obtained by Principal Components Analysis (PCA) (A) and PCA using NIPALS algorithm (B) for available concentrations of elements.

The variation in contribution to the PC of the individuals show similar behaviour between samples of roots and shoots from the plants with exception of the Neves Corvo samples where PC2 (As and Cu) and PC3 (Pb) of shoots behave differently than PC1 (Ca, Fe, Mg, Mn, Ni, K and Zn) and the roots of the same plants (Fig. 6). Similar groups were identified on the PC1 of soils available fractions and plants, although not always in the same sites. In the case of the available fraction of the soils, those are more related with Neves Corvo mine area. Whereas in case of the plants those are more related with Brancanes mine area. There is a strong correlation for Caveira area between Pb concentration in the soils available fraction and in the plants (shoots and roots). When observing individual contributions (not presented), all samples collected in Caveira are significantly more correlated with Pb than with other chemical elements, with special contribution of the sample Cav5. The exception is the total concentration of the elements in soils which have different behaviour. This different behaviour is expected, because total fraction of the elements in soils may not be entirely available for plant uptake and it differs depending on the chemical element (Fig. 6).

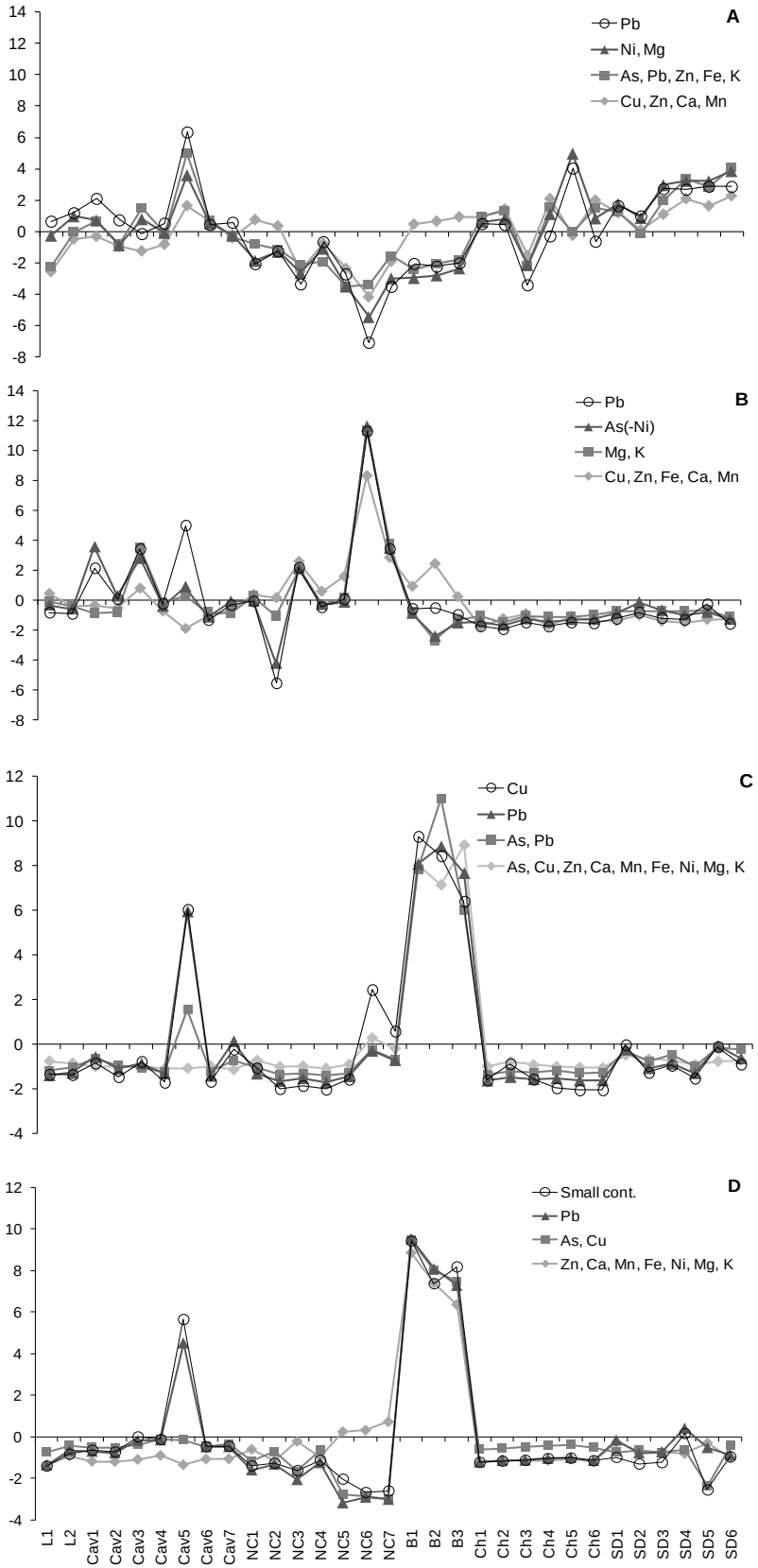

Fig. 6 Calculation determined by the prediction model (Q^2cumul) of the four principal components of the PCs of the individuals concentrations for soil total fraction (A), soil available fraction (B), *Cistus ladanifer* roots (C) and *Cistus ladanifer* shoots (D). (B: Brancanes; Cav: Caveira; Ch: Chança; L: Lousal; NC: Neves Corvo; SD: São Domingos).

There is not much variation in the scores representation in the shoots and roots of *C. ladanifer* populations from Lousal (although two samples are not representative of the population) and also from Chança and São Domingos (Fig. 6). The distal samples of Neves Corvo also show, in general, the same behaviour than those referred to Chança and São Domingos areas. PCA predictions for scaled (preprocessed) data (not presented) confirm some of the previous observations for score axis where Pb is related with the Caveira samples. Neves Corvo and Brancanes plant shoots, specially samples from the last area, show strong variations and important contributions of the elements grouped in the PC1 (Ca, Fe, Mg, Mn, Ni, K and Zn). These results were never observed in previous studies of Brancanes and Neves Corvo (Batista et al., 2007), because comparison among different mines, including these ones, with PCA studies were only possible in this study, when a representative number of samples was finally reached.The similarity between correlations to the axis and variables of São Domingos, Chança and Lousal is a new observation based on the present PCA studies. PCA residuals for scaled data (not presented) show that distal samples of Neves Corvo, Lousal and Caveira samples (elements concentration in the soil available fractions) are relevant, therefore not included in predictions of the modelled PCs. The same is true for plants, especially roots of São Domingos, Brancanes and Lousal where a relevant contribution to the PCs was not included in the predictions of the PC models. This may explain the apparent comparison of the elements concentrations in plants from São Domingos and Chança. Previous studies (Abreu et al., 2012a,b; Batista et al., 2009; Santos et al., 2012) report the low availability of the chemical elements in São Domingos soils which can also explain those comparison. Batista el al. (2007) reported the low chemical elements plant uptake, possibly due to the natural attenuation that seems to occur in Brancanes areas and the consequent soil-plant relationship. However, with this set of studied samples and the comparison between the six mine sites there is a strong difference in the behaviour of samples collected in Brancanes compared with the other samples of soils and *C. ladanifer* plants collected in Caveira, Chança, Lousal, Neves Corvo and São Domingos mine areas. Total concentrations of the elements in soils show comparable results with the previous studies where elements concentrations in soils are independent of the uptake of the elements by plants and the differences observed between samples contributions to the PCs can be explained by the diversity of materials and concentrations of chemical elements in soils/spoils where these plants were developed.

CONCLUSIONS

Soils from the different mine areas (Brancanes, Caveira, Chança, Lousal, Neves Corvo and São Domingos) showed a great heterogeneity of the total concentrations of trace elements, which is related to the diversity of materials where soils were developed.

Independently of mining area, the high concentrations of the trace elements in soils did not induce toxicity for *C. ladanifer* plants. This species is able to grow in mining areas with soils presenting different levels of polymetallic contamination. *Cistus ladanifer* presented variations inter- and intra-populations concerning the accumulation and translocation of chemical elements. Whatever the mine area where the plants grew, nutrients were mainly translocated from roots to shoots while trace

elements were stored in roots (except Neves Corvo for As and Pb and São Domingos for As). Plants belonging to the populations from all mine areas were non-accumulators of As, Cu, Pb, Fe and Ni but they were Ca accumulator. São Domingos and Brancanes plants populations were Zn accumulators.

In general, the total concentrations of chemical elements in shoots and roots of *C. ladanifer* populations from Lousal, Chança and São Domingos did not present much variation as well as some samples of Neves Corvo. The behaviour of the Brancanes plants population, as well the chemical elements concentrations in the soils where these plants grew, presented strong when compared to the other mine areas, both for soils and plants. *Cistus ladanifer* plants from all studied populations collected in mining areas, except Brancanes, seem to belong to the same population cluster

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REFERENCES

Abreu, M.M., Magalhães, M.C.F., 2009. Phytostabilization of soils in mining areas. Case studies from Portugal. In: Aachen, L., Eichmann, P. (Eds) Soil remediation. Nova Science Publishers, Inc. New York, pp 297-344.

Abreu, M.M., Santos, E.S., Anjos, C., Magalhães, M.C.F., Nabais, C.,2009. Lead uptake capacity of *Cistus* plants growing in mining areas. Revista de Ciências Agrárias 31(1), 170-181.

Abreu, M.M., Santos, E.S., Fernandes, E., Batista, M.J., Ferreira, M., 2011. Accumulation and translocation of trace elements in C*istus ladanifer* L. from IPB Portuguese mining areas. Revista de Ciências Agrárias 34(2), 44-56.

Abreu, M.M., Santos, E.S., Fernandes, E., Magalhães, M.C.F., 2012a. Trace elements tolerance, accumulation and translocation in *Cistus populifolius*, *Cistus salviifolius* and their hybrid growing in polymetallic contaminated mine areas. Journal of Geochemical Exploration 123, 52-60.

Abreu, M.M., Santos, E.S., Ferreira, M., Magalhães, M.C.F., 2012b. *Cistus salviifolius* a promising species for mine wastes remediation. Journal of Geochemical Exploration 113, 86-93.

Activation Laboratories, 2013a. Code 1H – Au + 48. http://www.actlabs.com/page.aspx?page=506&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes. Accessed June 2013

Activation Laboratories, 2013b. Code 6 – Hydrogeochemistry – ICP/MS. Available in: http://www.actlabs.com/page.aspx?page=544&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes.Accessed June 2013

Activation Laboratories, 2013c. 2D - Vegetation Ash - ICP/MS. Available in: http://www.actlabs.com/page.aspx?page=538&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes.Accessed June 2013

Alvarenga, P.M., Araújo, M.F., Silva, J.A.L., 2004. Elemental uptake and root-leaves transfer in *Cistus ladanifer* L. growing in a contaminated pyrite mining area (Aljustrel-Portugal). Water, Air and Soil Pollution 152, 81-96.

Álvarez-Valero, A.M., Pérez-López, R., Matos, J., Capitán, M.A., Nieto, J.M., Sáez, R., Delgado, J., Caraballo, M., 2008. Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian

Peninsula): evidence from a chemical and mineralogical characterization. Environmental Geology 55(8), 1797-1809.

Batista, M.J., Abreu, M.M., Serrano Pinto, M., 2004. Comportamento do Arsénio (III) e (V) em dois Litossolos e em estevas na área mineira de Neves Corvo. Revista de Ciências Agrárias 37(1), 291-300.

Batista, M.J., Abreu, M.M., Serrano Pinto, M., 2007. Biogeochemistry in Neves Corvo mining region, Iberian Pyrite Belt, Portugal. Journal of Geochemical Exploration 92, 159-176.

Batista, M.J., Gonzalez-Fernandez, O., Abreu, M.M., Carvalho, L., 2009. Chemical elements variation in leaves with different development stages of *Cistus* plants from S. Domingos mine area, South Portugal. Proceedings VII Congresso Ibérico e X Congresso Nacional de Geoquímica 407-415. Available in: http://repositorio.lneg.pt/bitstream/10400.9/740/1/33810.pdf. Accessed June 2013

CCME – Canada Council of Ministers of the Environment, 2007. Canadian soil quality guidelines for the protection of environmental and human health: Summary tables (updated September, 2007). CCME, Winnipeg, Canada

Chopin, E.I.B., Alloway, B.J., 2007. Distribution and mobility of trace elements in soils and vegetation around the mining and smelting areas of Tharsis, Riotinto and Huelva, Iberian pyrite belt, SW Spain. Water, Air and Soil Pollution 182, 245-261.

de la Fuente, V., Rufo, L., Rodríguez, N., Amils, R., Zuluaga, J., 2010. Metal accumulation screening of the Río Tinto Flora (Huelva, Spain). Biological Trace Element Research 134, 318-341.

Farago, M., Cole, M., Xiao, X.E., Vaz, M.C., 1992. Preliminary assessment of metal bioavailability to plants in the Neves Corvo area of Portugal. Chemical Speciation & Bioavailability 4(1), 19B27.

Feng, M.H., Shan, X.Q., Zhang, S.Z., Wen, B., 2005. A comparison of the rhizosphere-based method with DTPA, EDTA, CaCl2, and NaNO3 extraction methods for prediction of bioavailability of metals in soil to barley. Environmental Pollution 137, 231-240.

Freitas, H., Prasad, M.N.V., Pratas, J., 2004. Plant community tolerant to trace elements growing on degraded soils of São Domingos mine in the south east of Portugal: environmental implications. Environment International 30, 65-72.

Hill, T., Lewicki, P., 2007. STATISTICS: Methods and Applications. StatSoft, Tulsa, OK.

INMG, 1990. O clima de Portugal. Normais climatológicas da região de "Alentejo e Algarve" correspondentes a 1951-1980. Lisboa, Instituto Nacional de Meteorologia e Geofísica, 98 p. (Fascículo XLIX, Vol 4 – 4ª Região)

IUSS Working Group WRB, 2007. World reference base for soil resources 2006 (first update 2007). World Soil Resources Reports No. 103, Rome, FAO

Kabata-Pendias, A., 2011. Trace elements in soils and plants. 4th edition.CRC Press. Taylor & Francis Group, Boca Raton.

Kidd, P.S., Díez, J., Monterroso Martínez, C., 2004. Tolerance and bioaccumulation of heavy metals in five populations of *Cistus ladanifer* L. subsp. *ladanifer*. Plant and Soil 258, 189-205.

Lázaro, J.D., Kidd, P.S., Martínez, C.M., 2006. A phytogeochemical study of the Trás-os-Montes region (NE Portugal): Possible species for plant-based soil remediation technologies. Science of the Total Environment 354, 265-277.

Matos, J.X., Martins, L.P., 2006a. Reabilitação ambiental de áreas mineiras do sector português da Faixa Piritosa Ibérica: estado da arte e prespectivas futuras. Boletin Geológico y Minero 117, 289-304.

Matos, J.X., Martins, L.P., 2006b. Iberian Pyrite Belt Mining Region.Regional study of the Portuguese Sector.Noth East South West INTERREG IIIC. European Network of Mining Regions.INETI, Lisboa.

Matos, J.X., Martins, L.P., Oliveira, J.T., Pereira, Z., Batista, M.J., Quental, L., 2008. Rota da pirite no sector português da Faixa Piritosa Ibérica, desafios para um desenvolvimento sustentado do turismo geológico e mineiro. In: Paul Carrion (Ed.), Rutas Minerales en Iberoamérica. Projecto RUMYS, programa CYTED (136-155). Esc. Sup. Politécnica del Litoral, Guayaquil, Equador.

Murciego, A.M., Sánchez, A.G., González, M.A.R., Gil, E.P., Gordillo, C.T., Fernández, J.C., Triguero, T.B., 2007. Antimony distribution and mobility in topsoils and plants (*Cytisus striatus*, *Cistus ladanifer* and *Dittrichia viscosa*) from polluted Sb-mining areas in Extremadura (Spain). Environmental Pollution 145, 15-21.

Pérez-López, R., Álvarez-Valero, A.M., Nieto, J.M., Sáez, R., Matos, J.X., 2008. Use of sequential extraction procedure for assessing the environmental impact at regional scale of the São Domingos Mine (Iberian Pyrite Belt). Applied Geochemistry 23, 3452-3463.

Póvoas, I., Barral, M.F., 1992. Métodos de análise de solos. Comunicações do Instituto de Investigação Científica Tropical. Serie Ciências Agrárias 10, Lisboa

Pratas, J., Prasad, M.N.V., Freitas, H., Conde, L., 2005. Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation. Journal of Geochemical Exploration 85, 99-107.

Quental, L., Bourguignon, A., Sousa, A.J., Batista, M.J., Brito, M.G., Tavares, T., Abreu, M.M., Vairinho, M., Cottard, F., 2002. MINEO Southern Europe environment test site. Contamination impact mapping and modelling - Final Report. Assessing and monitoring the environmental impact of mining activities in Europe using advanced Earth Observation Techniques (MINEO) 5yth FP-IST-1999-10337

Reglero, M.M., Monsalve-González, L., Taggart, M.A., Mateo, R., 2008. Transfer of metals to plants and red deer in an old lead mining area in Spain. Science of the Total Environment 406, 287-297.

Risvic, H., 2007. Principal Component Analysis (PCA) & NIPALS algorithm. Available in: http://folk.uio.no/henninri/pca_module/pca_nipals.pdf

Santos, E.S., Abreu, M.M., Nabais, C., Saraiva, J., 2009 Trace elements and activity of antioxidative enzymes in *Cistus ladanifer* L. growing on an abandoned mine area. Ecotoxicology 18, 860-868.

Santos, E.S., Abreu, M.M., Nabais, C., Magalhães, M.C.F., 2012. Trace element distribution in soils developed on *gossan* mine wastes and *Cistus ladanifer* L. tolerance and bioaccumulation. Journal of Geochemical Exploration 123, 45-51.

Santos, E.S., Nabais, C., Abreu, M.M., 2013. Adaptabilidade ecofisiológica de diferentes populações de *Cistus ladanifer* L. do sul de Portugal. STUDIA – Scientiæ Rerum Diffusio: Suplemento Temático IV Seminário Luso-brasileiro em Ciências do Ambiente e Empresariais, 6 pp

Srivastava, P.C., Gupta, U.C., 1996. Trace elements in crop production. Science Publichers Inc, USA

Wong, M.H., 2003. Ecological restoration of mine degraded soils with emphasis on metal contaminated soils. Chemosphere 50, 775-780.

Zhang, W., Cai, Y., Tu, C., Ma, L.Q., 2002 Arsenic speciation and distribution in an arsenic hyperaccumulating plant. Science of the Total Environment 300, 167-177.

3. CISTUS LADANIFER PHYTOSTABILIZING SOILS CONTAMINATED WITH NON-ESSENTIAL CHEMICAL ELEMENTS

ABSTRACT

Cistus ladanifer L. is one of the spontaneous species from Iberian Pyrite Belt (IPB) considered promising for phytostabilisation of mining areas. Although the plant–soil relationships of some potentially hazardous elements (*e.g.* As, Pb, Cu and Zn) are known, for other elements also potentially hazardous and non-essential this information is scarce. However, in soils with multielemental contamination and for landscape rehabilitation processes, these elements should also be considered. The aim of this investigation was to evaluate the potential of C. ladanifer in the phytostabilisation of the soils, developed on different contaminated substrata (*e.g.* mine wastes, and schists and greywackes or their mixtures) and containing non-essential elements (Al, Ag, Ba, Bi, Cd, Sb and Sr), from three Portuguese IPB mining areas (Caveira, Lousal and São Domingos). The uptake, accumulation and translocation of these non-essential elements and their influence on the concentrations of beneficial elements (Co, Na and Se) and major nutrients were studied.

Multielemental contaminated soils from the three mining areas had high total concentrations of some non-essential elements (Ba and Sb for the three mines and Se for Caveira and São Domingos). Soil available fraction of the studied elements were usually small (< 8.6 % of the total concentration) except for Cd whose available fraction was between 10 and 100 % of the total concentration (< 0.3−1.3 mg Cd/kg). In general, *C. ladanifer* showed significant uptake (biological absorption coefficients indicating strong and intensive uptake) and translocation (translocation coefficients > 1) of the studied elements (even the non-essentials), but small accumulation in the shoots (mg/kg − Ag, Bi, Sb and Se: < 1.6; Ba, Cd, Co and Sr: 0.05−37.8; Al and Na: 49−2503), independently of the mine area. Elemental concentrations in the shoots were below the limit values indicated for phytotoxicity and toxicity for domestic animals intake. Although the statistical negative influence of Ag, Sb and Sr on the concentrations of the beneficial elements (Na) and nutrients (Ca and Fe) in roots or shoots had been obtained, no visual symptoms was observed in the plants. *Cistus ladanifer* plants from the studied populations can be considered non-accumulators and excluders of Al, Ag, Ba, Bi, Sr and Sb (soil-plant transfer coefficient << 1) and beneficial elements. Although plants from São Domingos and, in some cases, from Caveira can be accumulators of Cd, the concentrations of Cd in the shoots were small (< 0.2 mg/kg) and did not represent any environmental risk. Due to its adaptability, tolerance and standard plant behaviours in the three mining areas, *C. ladanifer* will be a good choice for phytostabilisation of soils contaminated with non-essential elements.

KEYWORDS Autochthonous vegetation • Portuguese Iberian Pyrite Belt • São Domingos mine •; Soil rehabilitation

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INTRODUCTION

Since pre-roman period until the last decades of the twentieth century, the mining activity in the Iberian Pyrite Belt (IPB), associated to the ore extraction and/or transformation, was developed without controlled rules for minimization of environmental or health risks. In addition, several mining areas had significant environmental impacts related to the great volumes of different tailings dispersed irregularly along the mine areas; high total concentrations of trace elements in the tailings, soils, sediments and waters; leaching of potentially hazardous elements for adjacent areas by water or wind erosion; and low production of biomass and biodiversity (Abreu et al., 2010; Álvarez-Valero et al., 2008; Matos and Martins, 2006; Pérez-Lopez et al., 2008). According to the above referred authors, the abandoned mines from IPB have additional environmental problems because some of their mine wastes are composed of reactive minerals (sulfides), which generate acid mine drainage.

In the European Community, the mining accidents that occurred between 1966 and 2000 (Aberfan in Wales at 1966; Aznalcóllar in Spain at 1986; Baia Mare in Romania at 2000, etc.) highlighted the need for major reforms in mining laws (Scanell, 2012). Nowadays, the European Community Environmental Authorities recognize the requirement to minimize the environmental impact of mining areas and their wastes, as well as their rehabilitation after closure (Directive 2006/21/EC). Nevertheless, other European Directives (*e.g.* Council Directives 2009/147/EC and 92/43/EEC) focus the importance of the environmental conservation as a whole, or of some key biodiversity groups (*e.g.* birds) or habitats.

In Portugal, few research programs have been developed on abandoned mines from Portuguese IPB aiming to contribute to their environmental assessment and monitoring of the contamination (*e.g.* Matos and Martins, 2006; Quental et al., 2002; Oliveira et al., 2013; Reis et al., 2012). Whereas active mines can have efficient procedures of environmental management, the rehabilitation programs of the abandoned mines, generally focus on public safety.

The rehabilitation of abandoned mines in the IPB must embrace ecological and economical approaches. Phytotechnologies are emerging, and are successful *in situ* tools to remediate contaminated soils due to their lower cost-effective and environmental impact as well as easier implementation in larger areas, compared to conventional *ex situ* engineering methods. Among the *in situ* technologies, phytostabilisation is considered the most suitable for rehabilitation of different mine wastes or soils developed on mine wastes with multielemental contamination and located in Mediterranean conditions (Abreu and Magalhães, 2009; Mendez and Maier, 2008; Sheoran et al., 2010). Although the total concentrations of trace elements did not change with the presence of plants, the above referred authors mentioned that the establishment of vegetation cover leads to landscape and soil functions improvement, increase of biodiversity, control of the water and wind erosion and of the contamination, by reducing trace elements availability and leaching in the soils/tailings.

At medium-long term, the success of the phytostabilisation and ecological succession in the mining areas depends on the knowledge of the characteristics of spoils and contaminated soils, rhizosphere processes as well as the behaviour of spontaneous, autochthones and pioneer plants (*e.g.* uptake, translocation, accumulation, chelation) (Abreu and Magalhães, 2009; Mendez and Maier, 2008; Pilon-

Smits, 2005). These plant populations are usually specific ecotypes well adapted to mining conditions (metallophytes) and, in Mediterranean conditions also to drought and nutritional stresses. The ecological behaviour of distinct plant species from IPB mining areas has been studied by several authors (*e.g.* Abreu et al., 2012a,b; Anawar et al., 2011; Batista et al., 2007; Chopin and Alloway, 2007; de la Fuente et al., 2010; Freitas et al., 2004).

Cistus ladanifer L. is one of the spontaneous species considered promising for phytostabilisation of mining areas with multielemental contamination (Abreu et al., 2011; Alvarenga et al., 2004; Anawar et al., 2011; Freitas et al., 2004; Lázaro et al., 2006; Murciego et al., 2007; Pratas et al., 2005; Santos et al., 2012). However, the majority of the studies in the field with this species (Abreu et al., 2011; Alvarenga et al., 2004; Anawar et al., 2011; Batista et al., 2013; Casado et al., 2007; Durães et al., 2015; Freitas et al., 2004; Lázaro et al., 2006; Murciego et al., 2007; Pratas et al., 2005; Santos et al., 2012, 2014a) focus, mainly, ecological behaviour and plant–soil relationships of some potentially hazardous elements (*e.g.* As, Cu, Pb, Zn) while for other potentially hazardous and non-essential elements (*e.g.* Al, Ag, Ba, Bi, Se) this information is scarce. It is also important to emphasize that the knowledge about the plant behaviour regarding these chemical elements, in different substrata and mining conditions, is scarce despite of its importance for the performance improvement of global rehabilitation programs.

Independently of the concentrations of elements in soils, *C. ladanifer* develops several tolerance mechanisms, which allow its adaptability to contaminated environments. Examples of this adaptability mechanisms are: trace elements immobilization in roots, accumulation in senescent leaves, maintenance of low concentrations of trace elements in young leaves appearing in Spring, presence of effective antioxidant enzyme-based defence systems, and translocation of N to young leaves, where it is reused, during the senescence (Abreu et al., 2011; Alvarenga et al., 2004; Batista et al., 2013; Durães et al., 2015; Santos et al., 2009, 2012, 2013, 2014a).

The aim of this investigation was to evaluate the potential of *C. ladanifer* in the phytostabilisation of the soils, developed on different substrata (mine wastes, and schists and greywackes or their mixtures) and containing non-essential elements (Al, Ag, Ba, Bi, Cd, Sb, and Sr), from three Portuguese IPB mining areas (Caveira, Lousal, and São Domingos). The uptake, accumulation and translocation of these non-essential elements, and their influence on the concentrations of major nutrients and beneficial elements (Co, Na and Se) in *C. ladanifer* were studied.

MATERIALS AND METHODS

Site characterization

Caveira, Lousal and São Domingos mining areas are included in the Portuguese IPB, Alentejo region, where the climate is typically Mediterranean (semiarid mesothermic). The Caveira and Lousal mines are located in the northwest region of the IPB, approximately 12 km away from each other, while São Domingos is in the northern sector of the IPB (about 120 km far from Caveira) (Fig. 1). According to Matos and Matins (2006), the massive sulfide deposits in the three mines were classified as medium (5−50 Tg) being exploited, approximately, in the same time period.

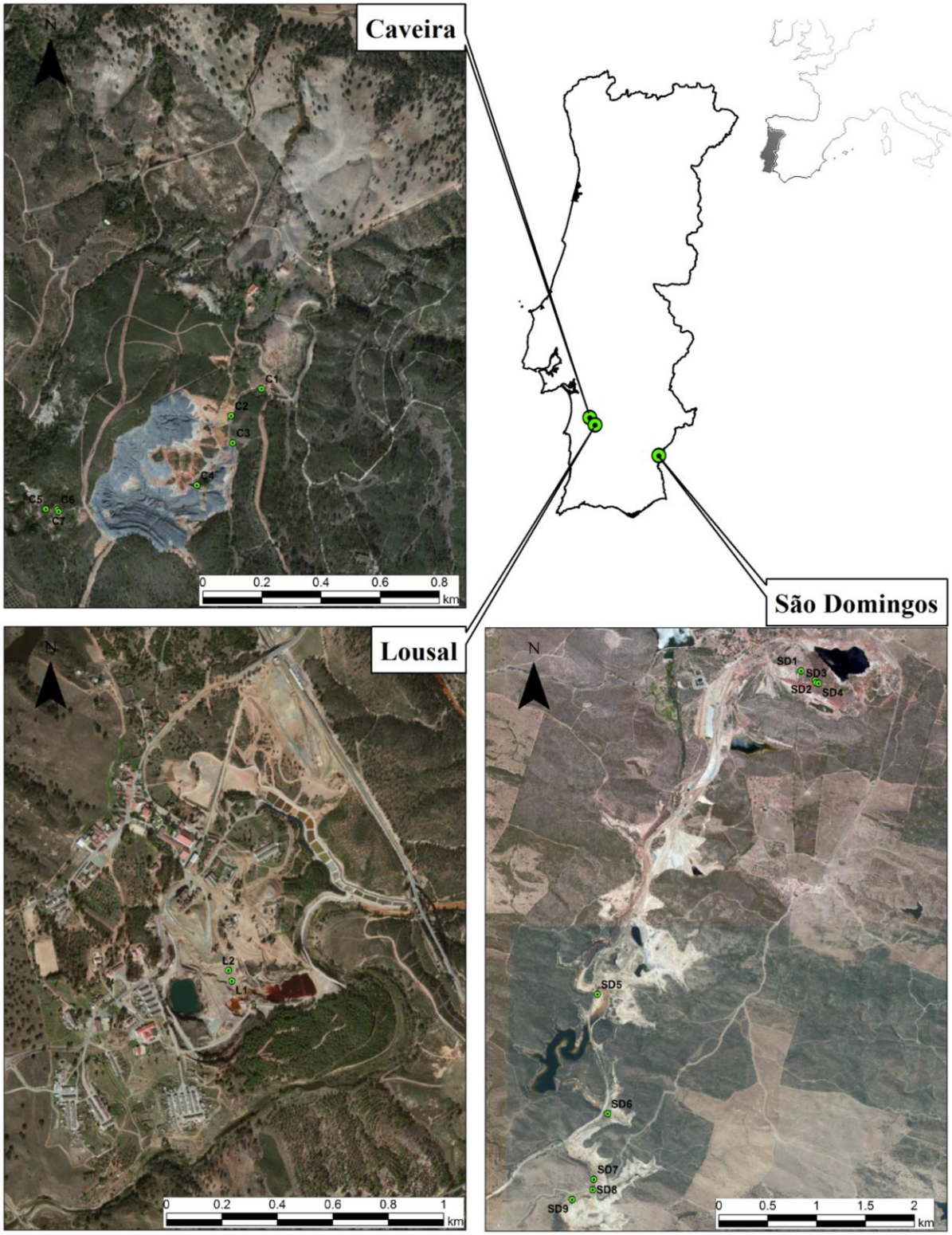

Fig. 1 Location of the studied mining areas in the Portuguese Iberian Pyrite Belt (Cav: Caveira; L: Lousal; SD: São Domingos) and respective sampling areas.

During Roman times, massive sulfide deposits and/or *gossan* from the Caveira and São Domingos were intensively exploited. The modern exploitation started in the XIXth century (Caveira: 1854, Lousal: 1900; São Domingos: 1857) ceasing between 1960 and 1980 with the abandonment of the

mines (Matos and Martins, 2006; Matos et al., 2008; Quental et al., 2002). The mine closures led to social and economic degradation in the region.

High level of environmental hazard was reported to the three mine areas taking into account the dimension of the areas affected by tailings, the volume of spoils and tailings, the multielemental contamination, and the available concentrations of trace elements in the different mine wastes/soils (Álvarez-Valero et al., 2008; Matos and Martins, 2006; Pérez-López et al., 2008).

Field sampling

Eighteen sampling areas with ≈10 m^2 each were selected (seven in Caveira, two in Lousal and nine in São Domingos; Fig. 1). These areas included representative soils where *C. ladanifer* is a dominant species growing, together or not, with other species of Cistaceae and Ericaceae families and, in Caveira mine, with sparse tree cover of *Quercus suber* L.. The soils from Caveira and São Domingos are classified as Spolic Technosol Toxic (IUSS Working Group WRB, 2007) while Lousal soils are Lithic Leptosol (IUSS Working Group WRB, 2007) influenced by adjacent tailings and/or acid mine drainage. Soil properties depended on materials where soils were developed presenting, in general, pH values considered moderately acid or acid, low electrical conductivity (except in Lousal) and variable fertility (Table 1; Santos et al., 2014a).

Composite samples of soils and *C. ladanifer* (roots and shoots composed of leaves and twigs from at least three plants) were collected in the Spring of 2010 in each sampling area. Soil samples were collected in the surrounding of the *C. ladanifer* radicular system. Each soil sampling (≈3 kg) was a homogenate of soil samples from, at least, three trial points located around the plants' roots at 20 cm depth.

Table 1 Characterization of soils from Caveira, Lousal and São Domingos mining areas (geometric mean (minimum – maximum)) (Santos et al., 2014a).

	Caveira (*n* = 7)	**Lousal (*n* = 2)**	**São Domingos (*n* = 9)**
pH (H_2O)	4.7 (3.9 – 6.2)	4.2 (4.0 – 4.5)	4.2 (3.5 – 4.7)
EC (µS/cm)	179 (122 – 286)	1849 (2540 – 1343)	267 (130 – 603)
Organic C (g/kg)	33.3 (13.0 – 64.3)	4.9 (3.8 – 6.5)	15.1 (5.9 – 22.4)
Total N (g/kg)	0.5 (0.02 – 2.7)	0.6 (0.6 – 0.7)	1.2 (0.6 – 1.9)
Extractable K (g/kg)	129 (58.1 – 324)	42.3 (41.5 – 43.2)	71.7 (45.6 – 143)
Extractable P (mg/kg)	2.1 (<0.9 – 74.3)	1.3 (0.9 – 1.8)	<0.9 (<0.9 – 2.7)

Characterization of soils and plants

The soil samples were air-dried, homogenized and sieved. In the soil fraction < 2 mm was done a classical characterisation (Póvoas and Barral, 1992): pH and electrical conductivity (EC) in water suspension (1:2.5 m/V); organic C (Tinsley method − oxidation with dichromate; Tinsley, 1950); extractable P and K (Egner-Riehm method − ammonium lactate-acetic acid extractant; Egnér et al., 1960) and total N (Kjeldahl method).

Total concentrations of beneficial (Co, Na and Se) and non-essential elements (Al, Ag, Ba, Bi, Cd, Sb and Sr) in soils (fraction < 2 mm) were analysed in an international accredited laboratory using ICP and INAA, after digestion with a mixture of four acids (Actlabs, ISO/IEC 17025, Activation Laboratories, 2014a). The same elements were analysed by ICP-MS and ICP-OES (Activation Laboratories, 2014b) in soil extractable solution that simulates the rizosphere conditions (RHIZO solution; Feng et al., 2005).

Roots and shoots samples were washed with tap water and then with distilled water. The roots were still sonicated in distilled water for 30 min. Plant samples were dried at 40 ºC, homogenized and finely ground. In these samples were determined the concentrations of the same elements than in soils by ICP-MS, after ashing at 475 °C and nitric acid digestion (Activation Laboratories, 2014c).

Data analysis

Data were analysed by a one way ANOVA and the Duncan test ($p < 0.05$), using the statistical programme SPSS v18.0 for Windows, in order to identify differences among plant populations. Differences between concentrations of the elements in roots and shoots from the same population were analysed non-parametrically using Kruskal–Wallis ANOVA by Ranks test. For statistical purposes, the results below the detection limit were assumed as half of the detection limit while for the results in over range was considered the limit value of the range. Bivariate Pearson correlations were used to correlate the soil and plant characteristics ($r > 0.85$). Quality control of the analysis was made by analytical replicate samples, use of certified standard solutions and laboratory standards at the Activation Laboratories.

Three coefficients reported by Huang and Cunningham (1996), Brook (1998) and Perelman (1966) were calculated to evaluate the potential of C. ladanifer for phytostabilization purpose: Soil-plant transfer coefficient (TransferC = [total shoots element]/[total soil element]), translocation coefficient (TranslC = [total shoots element]/[total roots element]), biological absorption coefficient (BAC = [total roots element]/[available soil element]). Soil-plant transfer and translocation coefficients have been considered as key parameters of the possible use of plants for phytostabilisation purposes (Mendez and Maier, 2008).

Soil-plant transfer coefficient indicates the accumulation behaviour of the plant (the plants can be considered as accumulator (TransferC > 1) or non-accumulator/excluder of a specific element (TransferC < 1)). The translocation of an element from roots to shoots was evaluated by the translocation coefficient while uptake capacity was assessed by biological absorption coefficient.

According to Perelman (1966), BAC can be divided in five groups, which indicate the intensity of absorption by the roots (Intensive: 10 < BAC < 100; Strong: 1 < BAC < 10; Intermediate: 0.1 < BAC < 1; Weak: 0.01 < BAC < 0.1; Very weak: 0.001 < BAC < 0.01).

RESULTS AND DISCUSSION

Soil characteristics

Chemical characteristics of the soils present, in general, great heterogeneity, which is related to the properties of the mine wastes and host rock from which they were developed (Tables 1 and 2). The total concentrations of Al, Ag, Ba, Bi and Sb as well as beneficial elements (Na and Se) in soils from three mining areas did not present any significant differences, despite of the variability observed in Caveira and São Domingos. Soils from São Domingos presented the highest total concentrations of Cd and Sr, while the highest value for Co was measured in Lousal (Table 2).

Table 2 Total concentrations of chemical elements in soils from Caveira, Lousal and São Domingos mining areas (mean (minimum – maximum)).

	Caveira (*n* = 7)	**Lousal (*n* = 2)**	**São Domingos (*n* = 9)**
	Non essential elements (*mg/kg dry weight)*		
Al	63×10^{3a} ($42 \times 10^3 – 76 \times 10^3$)	80×10^{3a} ($70 \times 10^3 – 90 \times 10^3$)	56×10^{3a} ($17 \times 10^3 – 86 \times 10^3$)
Ag	19^a (2.5 – 61)	1.4^a (1.0 – 1.8)	4.7^a (1.2 – 15)
Ba	431^a (< 50 – 860)	730^a (700 – 760)	597^a (< 50 – 1100)
Bi	15^a (< 2 – 42)	2.5^a (< 2 – 4.0)	24^a (< 2 – 117)
Cd	0.3^b (0.3 – 0.6)	0.7^{ab} (0.6 – 0.7)	1.1^a (< 0.3 – 1.3)
Sb	204^a (23 – 533)	29^a (22 – 37)	204^a (55 – 496)
Sr	61^b (48 – 101)	109^{ab} (76 – 142)	191^a (83 – 329)
	Beneficial elements (*mg/kg dry weight)*		
Co	4^b (3 – 14)	21^a (16 – 25)	6^b (2 – 13)
Na	4.3×10^{3a} ($2.3 \times 10^3 – 7.1 \times 10^3$)	5.5×10^{3a} ($3.4 \times 10^3 – 7.5 \times 10^3$)	6.3×10^{3a} ($1.5 \times 10^3 – 12.0 \times 10^3$)
Se	3.0^a (< 3 – 9)	$< 3.0^a$	9.2^a (< 3 – 42)

Different letters in means data from the same row indicate significant differences ($p < 0.05$).

Total concentrations of all non-essential and beneficial elements in the studied soils were in the range of the values reported for several mine wastes and/or soils from the same mines (Anawar et al., 2011; Álvarez-Valero et al., 2008; Ferreira da Silva et al., 2005; Pérez-López et al., 2008; Reis et al., 2012). Soils from the three mine areas had total concentrations of Ba and Sb that exceeded, the maximum allowed values (MAV) proposed by the Canadian legislation (500 mg Ba/kg for residential/parkland use, and 20–40 mg Sb/kg depending on the land uses; CCME, 2007), while Caveira and São Domingos soils also exceeded the MAV for Se considering commercial/industrial use (3 mg/kg; CCME, 2007).

Although the total concentrations of the studied elements in these mine soils can be relatively high (Table 2), the soil available fractions were usually small in all the mine areas (< 8.6 % of the total concentration; Table 3).

Table 3 Concentrations of chemical elements in the available fraction of soils from Caveira, Lousal and São Domingos mining areas (mean (minimum – maximum)).

	Caveira (*n* = 7)	**Lousal (*n* = 2)**	**São Domingos (*n* = 9)**
	Non essential elements (*µg/kg dry weight)*		
Al	> 20 x 10^{3a}	> 20 x 10^{3a}	>20 x 10^{3a}
Ag	2.4^{a} (2.0 – 4.0)	< 2^{b}	1.6^{ab} (< 2.0 – 3.0)
Ba	2.6 x 10^{3a}* (1.2 x 10^{3} – > 4.0 x 10^{3})	0.6 x 10^{3b} (0.5 x 10^{3} – 0.6 x 10^{3})	1.3 x 10^{3b} (0.3 x 10^{3} – 2.2 x 10^{3})
Bi	9.6^{a} (3.0 – 20.0)	< 3^{b}	2.1^{b} (< 3 – 4.0)
Cd	39.9^{a} (4.1 – 159.0)	92.2^{a} (68.5 – 116.0)	32.3^{a} (10.3 – 68.2)
Sb	447.5^{a}* (30.5 – > 1000)	57.0^{b} (55.2 – 58.8)	113.1^{ab} (25.4 – 200.1)
Sr	1.4 x 10^{3ab}* (0.5 x 10^{3} – > 2.0 x 10^{3})	1.5 x 10^{3a}* (1.0 x 10^{3} – > 2.0 x 10^{3})	0.7 x 10^{3b} (0.3 x 10^{3} – 1.2 x 10^{3})
	Beneficial elements (*mg/kg dry weight)*		
Co	0.4^{b} (0.1 – 1.2)	1.5*^{a} (1 – > 2)	0.3^{b} (0.1 – 1.1)
Na	34.2^{b} (12.3 – 46.8)	105.5^{a} (85.0 – 126.0)	26.8^{b} (15.3 – 59.5)
Se	0.02^{a} (0.01 – 0.03)	0.04^{a} (0.03 – 0.05)	0.03^{a} (0.01 – 0.07)

*Estimated mean considering the over range value. Different letters in means data from the same row indicate significant differences ($p < 0.05$).

The same geochemical partitioning was reported for other chemical elements (*e.g.* As, Cu, Pb, Zn), extracted with the same aqueous solution, in soils from the same mine areas (Abreu et al., 2012a,b; Santos et al., 2014a). In the present study, Cd is the exception, varying its available fraction between 10 and 100 % of the total concentration in the three mine areas.

Regarding the concentrations of the elements in the available fraction (Table 3), Caveira soils had higher concentrations of non-essential elements than those in soils from Lousal (Ag, Ba, Bi and Sb) and São Domingos (Ba and Bi), whereas the concentrations of Co, Na and Sr in soils from Lousal reached the highest values. For Cd and Se, no significant differences among the three areas were observed for their concentrations in the soils available fraction.

In general, no correlations were obtained between the elemental concentrations in the soil available fraction and the pH, the $C_{organic}$ concentrations, and the total concentrations of the studied elements in Caveira and São Domingos soils. Bismuth was an exception because a correlation (r = 0.92) was found between its concentration in the available fraction and the total concentration in soils from Caveira.

Chemical elements in plants

Non-essential chemical elements

The concentrations of the studied chemical elements in the roots and shoots of the *C. ladanifer* plants from the three mining areas are given in Tables 4 and 5, respectively. Non-essential elements can be important environmental contaminants in mining areas causing often phytotoxicity. In the three mine areas, the uptake (evaluated by the biological absorption coefficient (BAC) calculation; Table 6) of Ag by *C. ladanifer* can be considered intense depending on the population. However, the uptake capacity of Al, Ba, Bi, Cd and Sr varied between strong and intensive (Table 6) according to the population (plants from São Domingos present mainly intensive uptake), the chemical element, and even the sampled plants from the same population. Besides these factors, the uptake rate can also depend on the soil properties, the rhizosphere effects, the roots system, and the stage of plant development (Abreu et al., 2014; Kabata-Pendias, 2011). C*istus ladanifer* ability to uptake the non-essential elements (except for Cd) were high, which according to Kabata-Pendias (2011) is not consistent with the tendency observed for the plants in general. On the contrary, Cd seems to be easily uptake by the *C. ladanifer* roots as also reported by Adriano (2001) for plants in general.

Aluminium, Ag, Ba, Bi, Cd and Sr were stored in roots having, in the majority of the plant samples, higher concentrations than in the available soil fraction, but not higher than the total soil concentrations. In some plants sampled in Caveira and São Domingos was still observed that Cd concentration in the roots were between 1 and 7-fold higher than the total concentration in the soils, suggesting a great uptake and root accumulation capacity of this metal by this species. Plant roots can change the pH values of the rhizosphere and exudate compounds that affect availability of the chemical elements and their uptake by the plant (Abreu et al., 2014; Pilon-Smits, 2005).

Although the uptake and roots storage of Al, Ag, Ba, Bi, Cd and Sr can reach large concentrations, the Sb had a distinct behaviour. Thus, independently of the mine area, the uptake of Sb seemed be

more or less restricted (uptake considered mainly intermediate; Table 6) and the concentrations of this element in the roots were lower than its concentration in the available soils fraction. This fact can suggest the existence of a tolerance mechanism (Kabata-Pendias, 2011). Nevertheless, although in São Domingos population have some plants with strong uptake of Sb (Table 6), no tendency between variation intrapopulation and the concentration of this element in the available soil fraction was observed.

Table 4 Concentrations of the chemical elements in *Cistus ladanifer* roots collected in Caveira, Lousal and São Domingos mining areas (mean (minimum – maximum)).

	Caveira (*n* = 7)	**Lousal (*n* = 2)**	**São Domingos (*n* = 9)**	**References**
	Non-essential elements (*mg/kg dry weight)*			
Al	189.6^{b} (94.6 – 271.0)	273.9^{ab} (254.0 – 293.8)	446.3^{a} (233.6 – 699.7)	< 100 – 1100$^{(1)}$
Ag	0.08^{a} (0.04 – 0.1)	0.05^{a} (0.05 – 0.06)	0.09^{a} (0.06 – 0.1)	
Ba	9.0^{a} (5.1 – 21.4)	2.7^{a} (2.3 – 3.1)	15.5^{a} (2.6 – 43.2)	
Bi	0.1^{a} (0.04 – 0.3)	0.02^{a} (0.01 – 0.03)	0.2^{a} (0.03 – 0.3)	
Cd	0.2^{b} (0.04 – 0.5)	0.4^{ab} (0.3 – 0.5)	0.8^{a} (0.4 – 1.6)	
Sb	0.3^{a} (0.04 – 0.7)	0.05^{a} (0.04 – 0.05)	0.1^{a} (0.06 – 0.3)	
Sr	15.3^{a} (5.7 – 29.8)	9.7^{a} (9.2 – 10.3)	14.4^{a} (8.0 – 24.2)	
	Beneficial elements (*mg/kg dry weight)*			
Co	0.3^{b} (0.2 – 0.8)	1.1^{a} (0.7 – 1.4)	0.7^{ab} (0.3 – 1.7)	< 100 – 100$^{(1)}$
Na	173.6^{a} (63.9 – 348.5)	212.1^{a} (187.6 – 236.5)	175.6^{a} (79.9 – 467.6)	
Se	0.2^{a} (0.1 – 0.2)	0.3^{a} (0.1 – 0.4)	0.3^{a} (0.2 – 0.5)	

$^{(1)}$Durães et al. (2015), roots from the Iberian Pyrite Belt (Aljustrel, Lousal and São Domingos mines).
Different letters in means data from the same row indicate significant differences ($p < 0.05$).

Comparing concentrations of non-essential elements in the roots, only for Al and Cd were observed significant differences among populations. However no correlations were obtained between concentrations of Al and Cd in roots and the soil concentrations of the same elements (total and/or available fraction). The higher roots concentrations of Cd in São Domingos plants, compared to other

populations, can be a consequence of the high absorption capacity of this element by the plants (Table 6).

Table 5 Concentrations of the chemical elements in *Cistus ladanifer* shoots collected in Caveira, Lousal and São Domingos mining areas (mean (minimum – maximum)).

	Caveira (*n* = 7)	**Lousal (*n* = 2)**	**São Domingos (*n* = 9)**	**References**
		Non-essential elements (*mg/kg dry weight)*		
Al	131.9[b] (115.4 – 165.1)	178.5[b] (49.0 – 307.9)	1070[a] (194.1 – 2503)	900 – 1100[(1)]
Ag	0.06[a] (0.05 – 0.1)	0.03[a] (0.01 – 0.05)	0.06[a] (0.02 – 0.1)	0.1[(3)]
Ba	19.4[a] (2.5 – 37.8)	1.7[a] (0.2 – 3.1)	5.7[a] (2.4 – 7.6)	
Bi	0.04[a] (0.01 – 0.1)	0.06[a] (0.01 – 0.1)	0.3[a] (0.08 – 0.8)	
Cd	0.2[b] (0.05 – 0.5)	0.3[b] (0.2 – 0.5)	2.4[a] (0.9 – 3.3)	0.4 – 0.5[(2a)] 0.1[(2b)]
Sb	0.09[a] (0.06 – 0.2)	0.05[a] (0.01 – 0.1)	0.3[a] (0.04 – 0.5)	0.1 – 3.7[(4)] 0.7 ± 0.2[(5)]
Sr	11.8[a] (3.0 – 20.9)	4.0[a] (1.4 – 6.6)	9.0[a] (6.9 – 11.8)	
		Beneficial elements (*mg/kg dry weight)*		
Co	1.3[b] (0.9 – 1.7)	2.3[ab] (1.0 – 3.5)	2.9[a] (1.3 – 4.0)	2.5 ± 0.1[(5)]
Na	354.0[a] (277.8 – 480.9)	208.2[a] (74.9 – 341.6)	538.9[a] (296.9 – 1389.2)	100 – 300[(1)] 793 ± 639[(6)]
Se	0.1[a] (<0.05 – 0.3)	0.1[a] (0.03 – 0.2)	0.3[a] (0.1 – 0.4)	0.2 – 0.4[(2a)] 0.2 – 0.4[(2b)] 14.6 ± 1.9[(5)]

[(1)]Durães et al. (2015), roots from the Iberian Pyrite Belt (Aljustrel, Lousal and São Domingos mines), [(2a)]Reglero et al. (2008), leaves from Horcajo and Navalmartina mining area; [(2b)]Reglero et al. (2008), leaves from non contaminated area; [(3)]Freitas et al. (2004), leaves from São Domingos mine; [(4)]Pratas et al. (2005), leaves from Sarzedas mine; [(5)]Anawar et al., 2011, leaves from São Domingos mine; [(6)]de la Fuente et al. (2010), aerial part from Rio Tinto mine.
Different letters in means data from the same row indicate significant differences ($p < 0.05$).

The concentrations of the studied non-essential elements in the roots of *C. ladanifer* did not influence the concentrations of nutrients in the same organ (Santos et al., 2014a). However, the Sr concentrations in roots collected in São Domingos can have an antagonism effect ($r = -0.92$) in the Fe concentrations in the same plant roots (Fe data in Santos et al., 2014a).

Table 6 Values of biological absorption coefficient (BAC = [total roots element]/[available soil element]) in *Cistus ladanifer* collected in Caveira, Lousal and São Domingos mining areas (minimum – maximum).

	Caveira (*n* = 7)	**Lousal (*n* = 2)**	**São Domingos (*n* = 9)**
		Non-essential elements	
Al	5 – 14	13 – 15	12 – 35
Ag	21 – 36	46 – 61	29 – 120
Ba	1 – 11	4 – 5	2 – 19
Bi	4 – 21	3 – 10	46 – 172
Cd	2 – 22	4 – 5	12 – 38
Sb	0.3 – 1.0	0.7 – 0.9	0.2 – 10
Sr	5 – 18	5 – 0	7 – 36
		Beneficial elements	
Co	0.1 – 4	0.7 – 0.8	0.3 – 9
Na	1 – 12	2 – 3	3 – 16
Se	4 – 18	5 – 10	5 – 27

Perelman (1966) − Intensive: 10 < BAC < 100; Strong: 1 < BAC < 10; Intermediate: 0.1 < BAC < 1; Weak: 0.01 < BAC < 0.1; Very weak: 0.001 < BAC < 0.01

In general, *C. ladanifer* from the three populations showed great capacity to translocate the non-essential elements (except Sr) from roots to shoots (Table 7), despite of the intrapopulation variability. Translocation of Al from roots to shoots in *C. ladanifer* was also reported by Durães et al. (2015).

Interpopulation variability in the translocation behaviour of some non-essential elements was also observed (Table 7). For instance, Caveira plants stored, mainly, Al, Bi, Sb and Sr in their roots while the same behaviour was observed only for Ag and Sr in São Domingos plants. The heterogeneity in the translocation behaviour can be related to the plant age and transpiration rate of the shrubs (Pilon-Smits, 2005). However, similar concentrations of non-essential elements (except Cd) in the roots and shoots from the same population were observed. Independently of the translocation behaviour, concentrations of the non-essential elements in the *C. ladanifer* shoots from the three populations did not reach phytotoxic levels (5–10 mg Ag/kg, 500 mg Ba/kg, 5–30 mg Cd/kg and 150 mg Sb/kg; Kabata-Pendias, 2011) and were below the maximum tolerable levels (MTL) for domestic animals (mg/kg − Al: 1000, Ag: 100, Ba: 100; Bi: 500-1000, Cd: 10, Sr: 2000; NCR, 2005). Only some plants growing in São Domingos mine area had Al concentrations in the shoots, between 1265 and 2503 mg/kg, which exceeded the MTL (NCR, 2005). However, C. ladanifer is not used as feed for the domestic animals.

Cistus ladanifer shoots from Caveira and São Domingos had higher Cd concentrations than in the roots (São Domingos: between 2 and 5-fold; Caveira: between 1 and 3-fold) (Tables 4 and 5), which agree with the high mobility of this element into the plants (Adriano, 2001; Kabata-Pendias, 2011).

Considerable translocation of Cd from roots to shoots was also obtained in *C. ladanifer* from different proveniences growing in hydroponic solutions (Kidd et al., 2004).

Table 7 Values of translocation coefficient (TranslC = [total shoots element]/[total roots element]) in *Cistus ladanifer* collected in Caveira, Lousal and São Domingos mining areas (geometric mean (minimum – maximum)).

	Caveira (*n* = 7)	**Lousal (*n* = 2)**	**São Domingos (*n* = 9)**
		Non-essential elements	
Al	0.7 (0.6 – 1.3)	0.2 – 1.0	1.7 (0.5 – 3.6)
Ag	1.0 (0.3 – 1.3)	0.2 – 1.0	0.6 (0.5 – 1.0)
Ba	1.7 (0.3 – 6.6)	0.1 – 1.0	1.0 (0.7 – 1.6)
Bi	0.4 (0.1– 1.1)	1.2 – 3.4	1.6 (0.3 – 7.0)
Cd	1.6 (0.9 – 2.6)	0.4 – 1.4	2.5 (1.6 – 4.7)
Sb	0.5 (0.1 – 2.3)	0.2 – 2.1	1.8 (0.4 – 10.3)
Sr	0.8 (0.5 – 1.1)	0.1 – 0.7	0.8 (0.4 – 1.5)
		Beneficial elements	
Co	4.7 (2.1 – 10.2)	0.7 – 4.9	4.8 (1.9 – 10.6)
Na	2.2 (0.8 – 4.6)	0.3 – 1.8	2.4 (0.7 – 7.2)
Se	1.1 (0.1– 1.8)	0.1 – 1.8	0.9 (0.5 – 2.2)

As for roots, only the concentrations of Al and Cd in the shoots were significantly different among populations. São Domingos plants showed the highest shoots concentrations of these elements, compared to the other populations, despite of the existence of similar concentrations of these metals in mine soils (total and available fraction (only for Cd)). The concentrations of the elements in plants are, in general, the result of a set of plant behaviours, like uptake, accumulation in roots, translocation from roots to shoots, tolerance and synergism/antagonism processes, and can also be independent of the concentrations of the elements in the soil (Kabata-Pendias, 2011). Some authors (Kidd et al., 2004; Lázaro et al., 2006; Santos et al., 2012, 2013) reported that the accumulation capacity of several elements (contaminants and nutrients) in *C. ladanifer* depends on element and population.

As far as we know, there are few data concerning the concentrations of non-essential elements in *C. ladanifer*, especially in the roots (Tables 4 and 5). Thus, in spite of the intrapopulation variability of concentrations of the studied elements in the roots and shoots of the same species growing in other contaminated and non-contaminated areas studied by other authors (Tables 4 and 5), they were, in general, higher than those obtained in the present study. Although concentrations of Ag and Sb in Caveira plant shoots were not considered phytotoxic for plants in general, these elements concentrations seem to influence negatively Ca concentrations (Santos et al., 2014a) in the same plant part ($r_{Ag} = -0.88$ and $r_{Sb} = -0.86$).

The calculated values of the soil-plant transfer coefficient indicated that, in the three populations, the *C. ladanifer* is non-accumulator and excluder of Al, Ag, Ba, Bi, Sr and Sb (TransferC << 1). Same result was reported for Al and *C. ladanifer* growing in the Iberian Pyrite Belt mines (Durães et al., 2015). An exception was observed in one sampling area from Caveira, where the available fraction of Ba corresponded to 12 % of the total concentration (the largest percentage obtained) and the plants were accumulators of this element. For Cd, all plants growing in São Domingos (1.1 < TransferC < 18.2) and some plants from Caveira can be considered as accumulators (0.2 < TransferC < 3.2). In spite of the accumulation and translocation behaviours of Ba and Cd, the concentrations of these elements in the shoots indicate that none *C. ladanifer* population is considered a hyperaccumulator.

Beneficial chemical elements

Beneficial chemical elements can promote the growth or be essential for some plant species. However, the function and concentration vary with element and plant species (Pilon-Smits et al., 2009; de Varennes, 2003). Some authors report significant growth stimulation for some species in presence of Na besides its function as osmoticum and counter-ion during long-distance transport (de Varennes, 2003; Pilon-Smits et al., 2009; Subbarao et al., 2003).

Concentrations of the beneficial elements in the studied *C. ladanifer* roots had slight variations (Table 4) and were higher than the available soil fractions of the same elements (Table 3). However, a general tendency of absorption and accumulation of these elements by the plants can be defined (Se < Co < Na).

The uptake of Na and Se (evaluated by the biological absorption coefficient (BAC) calculation) in plants from the three populations was strong-intense (Table 6). This fact can explain the no existence of significant differences among the concentrations of these elements in the roots (Table 4).

However, Co concentrations in the roots showed inter-populational variability (Table 4), which was not explained by the Co concentrations in the soils (Tables 2 and 3) as well as by the differences in the plants uptake behaviour. Caveira and Lousal populations showed intermediate uptake behaviour for Co while the Co uptake behaviour in the plants from São Domingos can be considered mainly high (Table 6).

As reported for nutrients (Santos et al., 2014a) and Na (Durães et al., 2015) in the same species, the majority of the studied *C. ladanifer* plants also translocated the beneficial elements from roots to shoots (Table 7). However, the distribution of these elements between roots and shoots depended on

element and population ($[Se]_{roots}$ = $[Se]_{shoots}$ for the three populations; ($[Co\ and\ Na]_{shoots}$ > $[Co\ and\ Na]_{roots}$ in São Domingos and Caveira plants; $[Co\ and\ Na]_{roots}$ = $[Co\ and\ Na]_{shoots}$ in Lousal plants).

The concentrations of beneficial elements in the shoots and roots presented the same statistical differences among the populations (similar concentrations of Na and Se among populations and Co concentrations higher in plants from Lousal and São Domingos than in Caveira; Tables 4 and 5). No clear tendency between the variability intra- and inter-population in the shoots and the concentrations of elements in the soils (total and soil available fraction) was observed. Concerning the plants translocation and uptake behaviours, for the same elements, also no clear tendency was observed. The variations that can be observed in the plant behaviours can be related to plant response to other elements or to the chemical elements interactions (synergisms or antagonisms), as well as ecophysiological characteristics of each population.

Shoots of *C. ladanifer* collected in non-contaminated areas as well as in other mining areas showed a wide range of beneficial elements concentrations, being, in some cases, the results obtained in the present study (Table 5 and references therein) within the same range. Nonetheless, concentrations of Co and Se in the shoots from the three populations were in the range considered sufficient and normal (0.02−1 mg Co/kg, 0.01–2 mg Se/kg; Kabata-Pendias, 2011) for plants in general. Moreover, concentrations of these elements in the shoots were also below the maximum tolerable levels for domestic animals (25−100 mg Co/kg and 3−5 mg Se/kg; NCR, 2005).

The three *C. ladanifer* populations were non-accumulators of Co, Na and Se (TransferC < 1). Durães et al. (2015) also found that *C. ladanifer* from Lousal, Aljustrel and São Domingos were non-accumulators of Na. In general, concentrations of non-essential elements in the shoots or roots did not influence negatively the concentrations of the beneficial elements in the same organs. However, the shoots from Caveira plants showed a possible negative effect between concentrations of Sr and Na (r = −0.89). Antagonism interaction between Na and Mn (r = −0.85, Mn data in Santos et al., 2014a) in the roots of the plants collected in São Domingos was also observed. Similar interaction between Na and Mn was also reported by Kabata-Pendias (2011) for the plants in general.

Design considerations for the use of Cistus ladanifer in phytostabilisation programs

Cistus ladanifer can be indicated as a suitable species in rehabilitation of several mining areas from IPB due to its capacity and adaptability to colonize soils developed on different substrata (mine wastes and/or host rocks), where co-exist contamination of Ba, Se and Sb (according to values indicated by CCME, 2007) as well as other potentially hazardous elements like As and Pb (Reis et al., 2012; Santos et al., 2012, 2014a). Moreover, *C. ladanifer* shows a good vegetative development and absence of visual signs of phytotoxicity and/or deficiency of any elements, independently of soils characteristics where the population grows.

This species still has several characteristics/behaviours, which according to Mendez and Maier (2008) are considered adequate to its use in phytostabilisation programs of soils/wastes contaminated with non-essential elements. In spite of the relative small biomass production and the slow growth of *C. ladanifer*, this species is a non-accumulator plant (indicated by the soil-plant transfer coefficient) of

non-essential elements, have high concentrations of these elements in the roots as well as high uptake (indicated by the biological absorption coefficient), but low concentrations in the shoots. Although the non-essential elements can be translocated from the roots to the shoots, the shoots concentrations are low, below phytotoxic limit, and do not pose any environmental risk by their intake.

Natural attenuation of the contamination has been observed in the contaminated soils of some mining areas as a consequence of spontaneous plant colonization (Abreu et al., 2011; Batista et al., 2007). However, natural colonization presents, over time, different plant density and success, which depends on physical and chemical characteristics of the mine soils/wastes and tailings as well as colonizing capacity of the plant species (Valente et al., 2012). This process is slow and can minimize the leaching and spread of contaminants at long-term, but not always at short-term (Burgos et al., 2013). According to the same authors, it is necessary a speedy assisted remediation.

The addition of amendments, namely organic and inorganic wastes, is essential to promote the germination, establishment, colonization and development of the pioneer species, like *C. ladanifer*, in contaminated environments with low organic matter and nutrients content, and lack of structure in the substrata materials (Abreu and Magalhães, 2009; Wong, 2003). The improvement of the spontaneous plants individual growth can densify the natural populations. In fact, Kidd et al. (2007) reported an enhanced in *C. ladanifer* shoot biomass growing in non-contaminated soils amended with sewage sludge. In *gossan* mine wastes amended with a mixture of organic and inorganic wastes, the germination and development of this species was significantly improved, without the increase of the majority of the potentially hazardous elements concentrations in the available fraction and leachates (Santos et al., 2014b, 2015). It has been observed a microbial activity increase in the amended *gossan* wastes with plant development (Santos et al., 2014b, 2015), which contributes to the biogeochemical cycling and pedogenesis.

The application of amendments mixtures composed of several organic/inorganic residues, instead of using a single waste, can be more efficient in the rehabilitation process of this type of mining areas (Kumpiene et al., 2008). Nevertheless, the mixtures of amendments and application rates should be adjusted to the characteristics of each soil/tailing in order to make a specific Technosol that promotes and maintains biogeochemical processes along the time (Macías et al., 2011).

Although organic/inorganic wastes (from Technosols or not) can supply N to plant/soil community, the majority of N comes from N-fixation and its mineralization. In order to self-sustain the phytostabilisation, the use of different tolerant and autochthones plants species is recommended because they can perform distinct functional roles in the habitat. Hence, the use of plants from the Fabaceae (Leguminosae) family and/or other N-fixing species within the plant community can be an advantage in the process (Ahmad et al., 2012; Wong, 2003) when using ecotypes isolated from metal(loids) contaminated soils. According to the same authors, the contaminants can reduce the growth and multiplication of *Rhizobia* and affect the steps involved in Leguminosae–Rhizobium symbiosis, what contributes to a lower N fixation.

To guarantee a similar ecological behaviour and tolerance to the co-existence of various stress conditions from mining areas, the use of seeds collected in contaminated areas in phytostabilisation programs of heavily contaminated soils is recommended.

There are many tangible and intangible economic opportunities (also designated as direct and indirect values) for phytostabilisation. However, due to its complexity, an accurate economic evaluation of this technology is very difficult. Several intangible benefits are known, (*e.g.* carbon sequestration, erosion control, landscape aesthetics), as well as more tangible ones, like decrease of the contamination in the water and surrounding soils and the improvement of soils characteristics. The economic valorisation of the improvement of the ecological functions and the identification of target groups, which benefit from the mine rehabilitation, is difficult to assess.

Phytostabilisation programs have low installation and maintenance costs compared to other remediation options. Several industrial or agro-industrial wastes can be included in a strategy for wastes valorisation, through the design of a Technosol, instead of end up in landfills (Macías et al., 2011). This technology can increase the incomes from non-productive contaminated soils if associated to species with economic value (Licht and Isebrands, 2005). Different options can be economically viable for *C. ladanifer*, as production of pellets, essential oils and extraction of compounds for pharmaceutical and cosmetic. However, it is essential the assessment of the environmental and public safety risk of these products derived from contaminated areas.

CONCLUSIONS

Soils developed on different substrata from Caveira, Lousal and São Domingos mining areas have multielemental contamination with high total concentrations of some non-essential elements (Ba and Sb for the three mine areas and Se for Caveira and São Domingos). However, the soil available fractions of non-essential elements were usually low what reduce the environmental risk.

Independently of the mine area and soil characteristics, *C. ladanifer* colonized the contaminated soils showing large capacity of tolerance and adaptability to limiting conditions for vegetation growth. According to the ecophysiological behaviours (uptake, translocation and accumulation) of this species that led to the great uptake and roots accumulation of non-essential elements but low shoots accumulation, and consequently low toxicity for domestic animals and even low intake risk, this species can be considered a good choice for phytostabilisation. Furthermore, its potential economic value can be a considerable income for implementation of this technology.

Caveira and São Domingos mines are in an abandoned state and have great areas affected by old mining processes, so phytostabilisation technology using autochthonous plant species (*e.g. C. ladanifer*) can be an attempt to reduce the costs of their rehabilitation.

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REFERENCES

Abreu, M.M., Magalhães, M.C.F., 2009. Phytostabilization of soils in mining areas. Case studies from Portugal. In: Aachen, L., Eichmann, P. (Eds.), Soil remediation. Nova Science Publishers, Inc. New York, pp. 297-344.

Abreu, M.M., Batista, M.J., Magalhães, M.C.F., Matos, J.X., 2010. Acid mine drainage in the Portuguese Iberian Pyrite Belt. In: Brock C.R. (Eds.), Mine drainage and related problems. Nova Science Publishers, Inc. New York, pp. 71-118.

Abreu, M.M., Santos, E.S., Fernandes, E., Batista, M.J., Ferreira, M., 2011. Accumulation and translocation of trace elements in *Cistus ladanifer* L. from IPB Portuguese mining areas. Revista de Ciências Agrárias 34(2), 44-56.

Abreu, M.M., Santos, E.S., Ferreira, M., Magalhães, M.C.F., 2012a. *Cistus salviifolius* a promising species for mine wastes remediation. Journal of Geochemical Exploration 113, 86-93.

Abreu, M.M., Santos, E.S., Fernandes, E., Magalhães M.C.F. 2012b. Trace elements tolerance, accumulation and translocation in *Cistus populifolius*, *Cistus salviifolius* and their hybrid growing in polymetallic contaminated mine areas. Journal of Geochemical Exploration 123, 52-60.

Abreu, M.M., Bech J., Carvalho, L.C., Santos, E.S., 2014. Potential hazardous elements fluxes from soil to plants and the food chain. In: Bini, C., Bech, J. (Eds), PHEs, environment and human health: potentially harmful elements in the environment and the impact on human health. Springer, Netherlands, pp. 309-337.

Activation Laboratories. 2014a. 1H – Total Digestion – ICP, INAA. Available in: www.actlabs.com/page.aspx?page=506&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes (Accessed September 2014).

Activation Laboratories. 2014b. 2D – Vegetation Ash - ICP/MS. Available in: www.actlabs.com/page.aspx?page=538&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes (Accessed September 2014).

Activation Laboratories, 2014c. Code 6 – Hydrogeochemistry – ICP/MS. Available in: http://www.actlabs.com/page.aspx?page=544&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes (Accessed September 2014).

Adriano, D.C., 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals, 2nd edition, Springer-Verlag, New York.

Ahmad, E., Zaidi, A., Khan, M.S., Oves, M., 2012. Heavy metal toxicity to symbiotic nitrogen-fixing microorganism and host legumes. In: Zaidi, A., Parvaze, W., Khan, A., Saghir M. (Eds.), Toxicity of heavy metals to legumes and bioremediation. Springer-Verlag, Wien.

Alvarenga, P.M., Araújo, M.F., Silva, J.A.L., 2004. Elemental uptake and root-leaves transfer in *Cistus ladanifer* L. growing in a contaminated pyrite mining area (Aljustrel-Portugal). Water, Air and Soil Pollution 152, 81-96.

Álvarez-Valero, A.M., Pérez-López, R., Matos, J., Capitán, M.A., Nieto, J.M., Sáez, R., Delgado, J., Caraballo, M., 2008. Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian Peninsula): evidence from a chemical and mineralogical characterization. Environmental Geology 55(8), 1797-1809.

Anawar, H.M., Freitas, M.C., N. Canha, Regina, I.S., 2011. Arsenic, antimony, and other trace element contamination in a mine tailings affected area and uptake by tolerant plant species. Environmental Geochemistry and Health 33, 353-362.

Batista, M.J., Abreu, M.M., Serrano Pinto, M., 2007. Biogeochemistry in Neves Corvo mining region, Iberian Pyrite Belt, Portugal. Journal of Geochemical Exploration 92, 159-176.

Batista, M.J., de Oliveira, D.P.S., Abreu, M.M., Locutura, J., Shepherd, T., Matos, J., Bel-Lan, A., Martins, L., 2013. Sources, background and enrichment of lead and other elements: Lower Guadiana River. Geoderma 193-194, 265-274.

Brooks, R.R., 1998. Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining. CAB International, Wallingford.

CCME - Canada Council of Ministers of the Environment, 2007. Canadian soil quality guidelines for the protection of environmental and human health: Summary tables (updated September, 2007). CCME, Winnipeg, Canada.

Casado, M., Anawar, H.M., Garcia-Sánchez, A., Santa Regina, I., 2007. Antimony and arsenic uptake by plants in an abandoned mining area. Communications in Soil Science and Plant Analysis 38, 1255-1275.

Chopin, E.I.B., Alloway, B.J., 2007. Distribution and mobility of trace elements in soils and vegetation around the mining and smelting areas of Tharsis, Riotinto and Huelva, Iberian pyrite belt, SW Spain. Water, Air and Soil Pollution 182, 245-261.

Burgos, P., Madejón, P., Madejón, E., Girón, I., Cabrera, F., Murillo, J.M., 2013. Natural remediation of an unremediated soil twelve years after a mine accident: Trace element mobility and plant composition. Journal of Environmental Management 114, 36-45.

de la Fuente, V., Rufo, L., Rodríguez, N., Amils, R., Zuluaga, J., 2010. Metal accumulation screening of the Río Tinto Flora (Huelva, Spain). Biological Trace Element Research 134, 318-341.

de Varennes, A., 2003. Produtividade dos Solos e Ambiente. Escolar Editora, Lisboa.

Directive 2006/21/EC of the European Parliament and of the Council of 15 March 2006 on the management of waste from extractive industries and amending Directive 2004/35/EC - Statement by the European Parliament, the Council and the Commission.

Directive 2009/147/EC of the European Parliament and of the Council of 30 November 2009 on the conservation of wild birds.

Directive 92/43/EEC of Council of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora.

Durães, N., Bobos, I., Ferreira da Silva, E., Dekayir, A., 2015. Copper, zinc and lead biogeochemistry in aquatic and land plants from the Iberian Pyrite Belt (Portugal) and north of Morocco mining areas. Environmental Science and Pollution Research 22(3), 2086-2105.

Egnér, H., Riehm, H., Domingo, W.R., 1960. Untersuchungen über die chemische bodenanalyse als grundlage für die beurteilung des nährstoffzustandes der böden, ii: chemische extraktionmethod zur phosphor-und kaliumbestimmung. Kungl. Lantbr. Högsk. Ann. 26, 199-215.

Feng, M.H., Shan, X.Q., Zhang, S.Z., Wen, B., 2005. A comparison of the rhizosphere-based method with DTPA, EDTA, $CaCl_2$, and $NaNO_3$ extraction methods for prediction of bioavailability of metals in soil to barley. Environmental Pollution 137, 231-240.

Ferreira da Silva, E., Cardoso Fonseca, E., Matos, J.X., Patinha, C., Reis, P., Santos Oliveira, J.M., 2005. The effect of unconfined mine tailings on the geochemistry of soils, sediments and surface waters of the Lousal area (Iberian Pyrite Belt, Southern Portugal). Degradation & Development 16, 213-228.

Freitas, H., Prasad, M.N.V., Pratas, J., 2004. Plant community tolerant to trace elements growing on degraded soils of São Domingos mine in the south east of Portugal: environmental implications. Environment International 30, 65-72.

Huang, J.W., Cunningham, S.D., 1996. Lead phytoextraction: Species variation in lead uptake and translocation. New Phytologist 134(1), 75-84.

IUSS Working Group WRB, 2007. World reference base for soil resources 2006 (first update 2007).World Soil Resources Reports No. 103, Rome, FAO.

Kabata-Pendias, A., 2011. Trace elements in soils and plants. CRC Press, Boca Raton.

Kidd, P.S., Díez, J., Monterroso Martínez, C., 2004. Tolerance and bioaccumulation of heavy metals in five populations of *Cistus ladanifer* L. subsp. *ladanifer*. Plant and Soil 258, 189-205.

Kidd, P.S., Domínguez-Rodríguez, M.J., Díez, J., Monterroso, C., 2007. Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. Chemosphere, 66, 1458-1467.

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments - a review. Waste Management 28, 215-225.

Lázaro, J.D., Kidd, P.S., Martínez, C.M., 2006. A phytogeochemical study of the Trás-os-Montes region (NE Portugal): possible species for plant-based soil remediation technologies. Science of the Total Environment 354, 265-277.

Licht, L.A., Isebrands, J.G. 2005. Linking phytoremediated pollutant removal to biomass economic opportunities. Biomass Bioenergy 28(2), 203-218.

Macías, F., Macías-García, F., Nieto, C., Verde, J.R., Pérez, C., Bao, M., Camps-Arbestain, M.. 2011. Gestión de residuos y cambio climático. In: Mosquera, M.E.L., Osés, M.J.S. (Eds.), Gestión de residuos orgánicos de uso agrícola. Servizo de Publicacións e Intercambio Científico de la Universidade de Santiago de Compostela, Santiago de Compostela.

Matos, J.X., Martins, L., 2006. Reabilitação ambiental de áreas mineiras do sector português da Faixa Piritosa Ibérica: estado da arte e perspectivas mineiras. IGME. Boletin Geológico y Minero 117, 289-304.

Matos, J.X., Martins, L.P., Oliveira, J.T., Pereira, Z., Batista, M.J., Quental, L., 2008. Rota da pirite no sector português da Faixa Piritosa Ibérica, desafios para um desenvolvimento sustentado do turismo geológico e mineiro. In: Carrion, P. (Eds.), Rutas Minerales en Iberoamérica. Projecto RUMYS, programa CYTED (136-155). Esc. Sup. Politécnica del Litoral, Guayaquil, Equador.

Mendez, M.O., Maier, R.M., 2008. Phytoremediation of mine tailings in temperate and arid environments. Reviews in Environmental Science and Biotechnology 7, 47-59.

Murciego, A.M., Sánchez, A.G., González, M.A.R., Gil, E.P., Gordillo, C.T., Fernández, J.C., Triguero, T.B., 2007. Antimony distribution and mobility in topsoils and plants (*Cytisus striatus*, *Cistus ladanifer* and *Dittrichia viscosa*) from polluted Sb-mining areas in Extremadura (Spain). Environmental Pollution 145, 15-21.

NCR - National Research Council, 2005. Mineral tolerance of animals. National Academies Press, Washington.

Oliveira, M., Ferreira, T., Relvas, J.M.R.S., Pinto, A.M.M., Pereira, Z., Matos, J.X., Fernandes, C., 2013. Lousal, Portugal: Património geológico e mineiro de uma antiga mina na Faixa Piritosa Ibérica. Proceedings XIV Congresso Património Geológio e Mineiro, 19 pp..

Perelman, A.I., 1966. The geochemistry of land areas in Russian. Izd Vish Shk, Moscow. In: Nagaraju, A., Karimulla, S., 2002**.** Accumulation of elements in plants and soils in and around Nellore Mica Belt, Andhra Pradesh, India – a biogeochemical study. Environmental Geology 41, 852-860.

Pérez-López, R., Álvarez-Valero, A.M., Nieto, J.M., Sáez, R., Matos, J.X., 2008. Use of sequential extraction procedure for assessing the environmental impact at regional scale of the São Domingos Mine (Iberian Pyrite Belt). Applied Geochemistry 23, 3452-3463.

Pilon-Smits E., 2005. Phytoremediation. Annual Review of Plant Biology 56, 15-39.

Pilon-Smits, E.A.H., Quinn, C.F., Tapken, W., Malagoli, M., Schiavon, M., 2009. Physiological functions of beneficial elements. Current Opinion in Plant Biology 12, 267-274.

Póvoas, I., Barral, M.F., 1992. Métodos de análise de solos. Comunicações do Instituto de Investigação Científica Tropical. Serie Ciências Agrárias 10, Lisboa.

Pratas, J., Prasad, M.N.V., Freitas, H., Conde, L., 2005. Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation. Journal of Geochemical Exploration 85, 99-107.

Quental, L., Bourguignon, A., Sousa, A.J., Batista, M.J., Brito, M.G., Tavares, T., Abreu, M.M., Vairinho, M., Cottard, F., 2002. MINEO Southern Europe environment test site. Contamination impact mapping and modelling - Final Report. Assessing and monitoring the environmental impact of mining activities in Europe using advanced Earth Observation Techniques (MINEO) 5yth FP-IST-1999-10337.

Reglero, M.M., Monsalve-González, L., Taggart, M.A., Mateo, R., 2008. Tranfer of metals to plants and red deer in an old lead mining area in Spain. Science of Total Environment 406, 287-297.

Reis, A.P., Ferreira da Silva, E., Cardoso Fonseca, E., Patinha, C., Barrosinho, C., Matos, J., 2012. Environmental assessment of the Caveira abandoned mine (southern Portugal): Part 1: characterization of metal contaminated soil. Soil and Sediment Contamination: An International Journal 21(2), 227-254.

Santos, E.S., Abreu, M.M, Nabais, C., Saraiva, J.A., 2009. Trace elements and activity of antioxidative enzymes in *Cistus ladanifer* L. growing on an abandoned mine area. Ecotoxicology 18, 860-868.

Santos, E.S., Abreu, M.M, Nabais, C., Magalhães, M.C.F., 2012. Trace elements distribution in soils developed on *gossan* mine wastes and *Cistus ladanifer* L. tolerance and bioaccumulation. Journal of Geochemical Exploration 123, 45-51.

Santos, E.S., Nabais, C., Abreu, M.M, 2013. Adaptabilidade ecofisiológica de diferentes populações de *Cistus ladanifer* L. do sul de Portugal. *STUDIA – Scientiæ Rerum Diffusio*: Suplemento Temático IV Seminário Luso-Brasileiro em Ciências do Ambiente e Empresariais, 6 pp.

Santos, E.S., Abreu, M.M, Macías, F., Batista, M.J., Magalhães, M.C.F., Fernandes, E., 2014a. Inter-population variation on the accumulation and translocation of potentially harmful chemical elements in *Cistus ladanifer* L. from Brancanes, Caveira, Chança, Lousal, Neves Corvo and São Domingos mines in the Portuguese Iberian Pyrite Belt. Journal of Soils and Sediments 14, 758-772.

Santos, E.S., Abreu, M.M, Macías, F., de Varennes, A., 2014b. Improvement of chemical and biological properties of *gossan* mine wastes following application of amendments and growth of *Cistus ladanifer* L.. Journal of Geochemical Exploration 147, 173-181.

Santos, E.S., Abreu, M.M, Macías, F., de Varennes, A., 2015. Chemical quality of leachates and enzimatic activities in Technosols with *gossan* and sulfide wastes from the São Domingos mine. Journal of Soils and Sediments 16(4), 1366-1382.

Scanell, Y., 2012. The regulation of mining and mining waste in the European Union. Journal of Energy and Environmental 177, 177-268.

Sheoran, V., Sheoran, A.S., and Poonia, P., 2010. Soil Reclamation of Abandoned Mine Land by Revegetation: A Review. International Journal of Soil, Sediment and Water 3(2), article 13, 21 pp..

Subbarao, G.V., Ito, O., Berry, W.L., and Wheeler, R.M., 2003. Sodium – a functional plant nutrient. Critical Reviews in Plant Sciences 22, 391-416.

Tinsley, J., 1950. The determination of organic carbon in soils by dichromate mixtures. Trans. 4th International Congress of Soil Science 1, 161-165.

Valente, T., Gomes, P., Pamplona, J., Torre, M.L. 2012. Natural stabilization of mine waste-dumps — Evolution of the vegetation cover in distinctive geochemical and mineralogical environments. Journal of Geochemical Exploration 123, 152-161.

Wong, M.H., 2003. Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. Chemosphere 50, 775-780.

4. MUTIELEMENTAL CONCENTRATION AND PHYSIOLOGICAL RESPONSES OF LAVANDULA PEDUNCULATA GROWING IN SOILS DEVELOPED ON DIFFERENT MINE WASTES

ABSTRACT

This study aimed to: i) evaluate the accumulation and translocation patterns of potentially hazardous elements into the *Lavandula pedunculata* and their influence in the concentrations of nutrients; and ii) compare some physiological responses associated with oxidative stress (concentration of chlorophylls (Chl*a*, Chl*b* and total), carotenoids, and total protein) and several components involved in tolerance mechanisms (concentrations of proline and acid-soluble thiols and total/specific activity of catalase (CAT) and superoxide dismutase (SOD)), in plants growing in soils with a multielemental contamination and non-contaminated. Composite samples of soils, developed on mine wastes and/or host rocks, and *L. pedunculata* (roots and shoots) were collected in São Domingos mine (SE of Portugal) and in a reference area with non-contaminated soils, Corte do Pinto, with the same climatic conditions.

São Domingos soils had high total concentrations of several hazardous elements (*e.g.* As and Pb) but their available fractions were small (mainly <5.8 % of the total). Translocation behaviour of elements was not clear according to the physiological importance of the elements. In general, plant shoots from São Domingos had the highest elements concentrations, but only As, Mn and Zn reached phytotoxic concentrations. Concentration of Chl*b* in shoots from São Domingos was higher than those from Corte do Pinto. No significant differences were obtained between concentrations of Chl*a*, total protein, proline and acid-soluble thiols in shoots collected in both areas, as well as SOD activity (total and specific) and specific CAT activity. Total CAT activity varied with population being lower in the shoots of the plants from São Domingos, but no correlation was obtained between this enzymatic activity and the concentrations of the studied elements in shoots.

Lavandula pedunculata plants are able to survive in soils developed on different mine wastes with multielemental contamination and low fertility showing no symptoms (visible and physiological) of phytotoxicity or deficiency.

KEYWORDS Iberian Pyrite Belt • Mutielemental contamination • Physiological responses • São Domingos mine

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INTRODUCTION

Over several decades and until the publication of Directive 2006/21/EC, which requires the landscape rehabilitation after mine closure, the mining activity in Portuguese Iberian Pyrite Belt (PIPB) was carried out without any environmental concerns. Over time, the extraction and processing of the ore produced different mining wastes which were disposed in an uncontrolled and irregularly way in all the mine areas (Matos and Martins, 2006). As a consequence, significant environmental problems were occurring (*e.g.* contamination of soils and waters and reduction of biodiversity), not only in the mining area but also in the adjacent areas, due to the chemical characteristics of the mine wastes in the PIPB (*e.g.* acid mine generation and high total concentrations of trace elements in tailings and leachates) (Matos and Martins, 2006; Abreu and Magalhães, 2009).

Mining areas from PIPB become extreme environments where coexist many stress factors for the plants: high total concentrations of potentially hazardous elements in the soils/mine wastes and leachates as well as soil water deficit and low soils/wastes pH and fertility. However, some tailings and soils developed on some mine wastes are naturally colonized by spontaneous vegetation, which improves the physical, chemical and biological characteristics of the substrata and, consequently, contributes to the pedogenesis and the natural rehabilitation of the area (Wong, 2003; Abreu and Magalhães, 2009). Although very few species occur only in the specific conditions of the mining areas (*e.g. Erica andevalensis* Cabezudo & Rivera), other species (*e.g.* several species from *Cistus* genus) are able to grow in contaminated and non-contaminated areas (Abreu et al., 2012a; Santos et al., 2012) being specific ecotypes. These tolerant ecotypes can be classified as obligate or facultative metallophytes having effective tolerance mechanisms (Nadgórska-Socha et al., 2013).

In stress conditions, like metal/metalloid toxicity, several physiological processes in the plants (e.g. photosynthesis and growth) can be affected due to an increase of the production of reactive oxygen species (ROS) and their accumulation in the cells. Nonetheless, plants have several antioxidative defence responses to scavenge ROS (Pang et al., 2003; Cao et al., 2004; Słomka et al., 2008; Nadgórska-Socha et al., 2013): produce or stimulate antioxidative enzymes (*e.g.* catalase – CAT and superoxide dismutase – SOD) and/or non-enzymatic components (*e.g.* acid-soluble thiols, carotenoids and proline), which remove and/or neutralize the ROS. Superoxide dismutase catalyses the transformation of ROS generating H_2O_2 (Cao et al., 2004), while CAT is involved in the H_2O_2 removal generated by SOD (Grant and Loake, 2000). Proline accumulation is a very effective mechanism of quencher of ROS and redox active metal ions binder but it also activates and protects some enzymes related to antioxidative defence (Teklić et al., 2008). Thiols are also capable of binding metal ions and forming non-toxic complexes with metals (Nadgórska-Socha et al., 2013).

Lavandula pedunculata (Mill.) Cav. (Sin. *L. sampaiana* (Rozeira) Rivas Mart., T.E. Díaz & Fern. Gonz and *L. stoechas* L. subsp. *pedunculata* (Mill.) Samp. Ex Rozeira) is one of the autochthones and spontaneous species identified in São Domingos mining area (Freitas et al., 2004a), an abandoned mining area from the Iberian Pyrite Belt (SE of Portugal), however the knowledge about its ecophysiological behaviour under contaminated conditions is scarce. This information about the interaction between stress factors, in this case the multielemental contamination, and physiological

processes can be useful in the design of a strategy for revegetation of mining areas (Nadgórska-Socha et al., 2013), namely production sustainability improvement and efficient agricultural zoning (Campostrini and Glenn, 2007).

This study aimed to: i) evaluate the accumulation and translocation patterns of potentially hazardous elements into *L. pedunculata* and their influence in the concentrations of the nutrients; and ii) compare some physiological responses associated with oxidative stress (concentration of chlorophylls, carotenoids and total protein) and several components involved in tolerance mechanisms (concentrations of proline and acid-soluble thiols and total/specific activity of catalase and superoxide dismutase) in plants growing in soils with a multielemental contamination (São Domingos mining area) and non-contaminated (Corte do Pinto) with the same climatic conditions.

MATERIALS AND METHODS

Site characterization

For this work were studied two sites located in Alentejo region (SE, Portugal): a contaminated area, São Domingos mining area (SD), and a reference area with non-contaminated soils − Corte do Pinto (CP). In both areas, L. pedunculata is a representative species in the vegetation community. The climate in these areas is typically Mediterranean (semiarid mesothermic − Thornthwaite classification). São Domingos is an old copper mine located in the PIBP, which has been abandoned since 1960. The first mining activities date from pre-roman period with Ag, Au and Cu exploitation, in the gossan. Then in modern times, exploitation occurred between the middle of XIX century until 60's, for Cu, S, Au, Pb and Zn in both the *gossan* and massive sulfides (Quental et al., 2002). During the several exploitation periods, large amounts of mining wastes were produced and spread, irregularly, along the mine area (Álvarez-Valero et al., 2008). The chemical characteristics of these mine wastes represent a high environmental risk (Pérez-López et al., 2008; Álvarez-Valero et al., 2008). The sampling areas are located within the mine area where soils are incipient and were developed on mixtures, in variable proportions, of different mine wastes and host rocks being classified as Spolic Technosol Toxic (IUSS Working Group WRB, 2007). In the mining area, there are also some soils developed on schists and greywackes (Lithic Leptosols; IUSS Working Group WRB, 2007), which are influenced by adjacent tailings and/or acid mine drainage.

Corte do Pinto area is located about 4 km to north of São Domingos mine. In the sampling areas, the soils were developed on schist and greywackes being classified as Lithic Leptosols (IUSS Working Group WRB, 2007).

Field sampling

Several sampling areas (six in SD mining area and three in CP), with ≈10 m^2 each, were selected to include representative soils where *L. pedunculata* was growing. The sampling was done in the spring of 2012 (April). In each sampling area, composite samples of soils (≈3 kg of homogenate of, at least, three trial points) were collected in the surrounding of the *L. pedunculata* radicular system (until

a maximum of 20 cm depth). At the same time and in the same sampling areas, composite samples of roots (only São Domingos population) and shoots (composed of leaves and twigs) of *L. pedunculata* plants (homogenate of more than five plants, depending on the plants development stage) were collected.

Soils characterization

The soil samples were air-dried, homogenised, and sieved. Classical characterization of soils (fraction < 2 mm) was carried out according to Póvoas and Barral (1992): pH and electrical conductivity (EC) in water suspension (1:2.5 *m*/*V*); organic carbon (Ströhlein method); extractable P and K (Egner-Riehm method) and total nitrogen (Kjeldahl method).

The total concentrations of Al, As, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, Pb, Sb, V and Zn in soils (fraction < 2 mm) were determined by ICP and INAA, after acid digestion with perchloric acid + nitric acid + hydrochloric acid + hydrofluoric acid (Actlabs ISO/IEC 17025; Activation Laboratories, 2015a).

The same elements were analysed in a soil extractable aqueous solution, which is composed of a mixture of organic acids (acetic acid + lactic acid + citric acid + malic acid + formic acid at 10 mmol/L for 16 h of agitation) that simulate rhizosphere conditions (RHIZO solution; Feng et al., 2005). These extractable soil solutions were analysed by ICP-MS and ICP-OES (Activation Laboratories, 2015b).

Plants characterization: multielemental composition

Roots from SD samples and shoot samples from both populations were washed with tap water and then with distilled water. The roots were still sonicated in distilled water for 30 min. Plant samples were dried at 40 ºC, homogenised and finely ground. The same elements than in soils were analysed by ICP-MS, after ashing (475 °C) over a 36 h period and nitric acid digestion (Activation Laboratories, 2015c).

Plants characterization: physiological analyses

After washing, shoot samples were frozen in liquid nitrogen and stored at −80 °C. Before the analysis, shoots were lyophilized, finely grounded and homogenised.

Pigments determination

The assay was based on MacKinney (1941). Shoot samples (0.1 g) were homogenised in 4 ml of 80 % acetone (*V*:*V*) using a homogeniser at 500 *g* for 15 min, and then these samples were left to macerate for 2 h at 4 °C. The extracts were centrifuged and the supernatant used for pigments determination. The concentrations of chlorophyll a (Chl*a*), chlorophyll b (Chl*b*), total chlorophyll (Chl*t*) and carotenoids were determined by UV-VIS spectrophotometry at 480, 645 and 663 nm using the equations described by MacKinney (1941) and Kirk and Allen (1965) for chlorophylls and carotenoids,

respectively. Acetone was used as the blank. Results were expressed as mg chlorophyll/g fresh weight and µmol carotenoids/g fresh weight.

Proline determination

The acid-ninhydrin method was used to determine the proline content (Bates et al., 1973). The free proline was extracted from 0.5 g of shoots in 5 ml of 3 % (*m*:*V*) aqueous sulfosalicylic acid. After centrifugation, the supernatant was mixed in 1:1:1 ratio with ninhydrin acid and glacial acetic acid and incubated at 100 °C for 1 h. The reaction mixture was placed on ice and extracted with 4 mL of toluene. The organic toluene phase containing the chromophore was separated and absorbance of the developed red colour was read at 520 nm. Toluene was used as the blank. Proline concentration was determined using a calibration curve and expressed as µg/g fresh weight.

Total acid-soluble thiols determination

Total acid-soluble thiols (–SH) was determined according to Ellman's method (Ellman, 1959). Shoot samples (0.1 g) were homogenised in 2 ml 5 % (*m*:*V*) sulfosalicylic acid, containing 6.3 mM DTPA (pH <1), at 0 °C. The homogenate was centrifuged at 10000 *g* for 15 min at 4 °C. The supernatants were collected and used for thiols determination. The supernatant (300 µl) was mixed with 630 µl of 0.5 M K_2HPO_4 (pH 7.5), and the absorbance measured after 2 min at 412 nm (30 °C). After addition of 25 µl of 5,50-dithiobis-2-nitrobenzoic acid (DTNB) solution (10 mM DTNB, 0.143 M K2HPO4, 6.3 mM DTPA, pH 7.5), the absorbance was again read at 412 nm after 2 min. Acid-soluble thiol concentrations were determined using the extinction coefficient (ε) of DTNB (14150 M^{-1} cm^{-1}) (Riddles, 1979). The results were expressed as µmol −SH/g fresh weight.

Total protein determination

Total protein was determined in the supernatant used for quantification of the enzymatic activity according to Bradford (1976). Protein concentration was determined using a calibration curve of bovine serum albumin (BSA) and expressed as mg/g fresh weight.

Enzyme extraction and activity quantification

The enzymatic extract was obtained from 0.1 g of shoots with 10 mL of a 50 mM phosphate buffer solution (pH 7.2) containing 1 mM of EDTA, 1 % (*m*:*V*) of polyvinylpolypyrrolidone (PVPP) and 1 M of NaCl (based on Santos et al., 2009). Extraction occurred for 15 min at 4 °C, with continuous agitation followed by centrifugation. The supernatant, constituting the enzymatic extract, was frozen and stored until further analysis. Substrate saturation and linear relation between enzyme activity and enzyme concentration was previously determined.

Quantification of catalase (CAT − EC 1.11.1.6) activity was based on Chance and Maehly (1955) and Wong and Whitaker (2003). Solutions of 0.1 M phosphate buffer (pH 7.0) and 200 mM of H_2O_2 were previously incubated at 25 °C. In quartz cuvettes, 800 µL of phosphate buffer, 320 µL of enzymatic extract and 80 µL of H_2O_2 were mixed. Reaction was started by the addition of H_2O_2. The decomposition of H_2O_2 was followed at 240 nm for 1 min and the slope of the linear portion of the curve relating absorbance at 240 nm with time was calculated (ΔAbs240 min^{-1}). The calculated slope was used to determine the CAT activity (µmol H_2O_2 min^{-1} g^{-1} shoots fresh weight) using the ε for H_2O_2 of 36.0 M^{-1} cm^{-1} (Verma and Dubey 2003). Specific enzymatic activity (µmol H_2O_2 min^{-1} mg^{-1} protein) was also determined taking into account the protein concentration obtained in the same extract (section 2.5.4).

Quantification of superoxide dismutase (SOD − EC 1.15.1.1) activity was based on the method described by Sun and Zigman (1977) and Khopde et al. (2001). Solutions of 0.1 M of sodium carbonate-hidrogenocarbonate buffer (pH 10.0) and 5 mM of epinephrine (pH 2.0) were incubated at 25 °C. In quartz cuvettes, 750 µL of buffer, 100−150 µL of sample and 150 µL of epinephrine were mixed. The enzymatic reaction was started by the addition of epinephrine. The increase of the absorbance was recorded at 320 nm for 1 min and the rate of epinephrine oxidation calculated by the slope (ΔAbs320 min^{-1}) of the linear portion of the curve relating absorbance at 320 nm with time. The rate of epinephrine auto-oxidation was calculated in the same conditions but without enzymatic extract. One unit of SOD was defined as the amount of enzyme required to reduce the epinephrine auto-oxidation rate by 50 %. Specific enzymatic activity (U/mg protein) was also determined taking into account the protein concentration obtained in the same extract (section 2.5.4).

Data analysis

Differences between *L. pedunculata* populations were analysed non-parametrically using Kruskal–Wallis ANOVA by Ranks test, with the statistical programme SPSS v18.0 for Windows. Bivariate Pearson correlations were used to correlate the soil and plant characteristics as well as the elements concentrations in the shoots and results of the physiological analyses (r > 0.90). For statistical purposes, the results below the detection limit were assumed as half of the detection limit while for results exceeding the quantification range was considered the quantification limit value.

The multielemental analyses (plants and soils) were done by Activation Laboratories which is accredited with ISO/IEC 17025 Quality System. Quality control of the other analysis was made by analytical duplicates samples (for each plant sample and physiological analysis) as well as the use of certified standard solutions and method reagent blank.

Soil-plant transfer and translocation coefficients were calculated (Abreu et al., 2012a). Soil-plant transfer coefficient (TransferC = [total shoots element]/[total soil element]) indicates if the plants are considered as accumulator (TransferC > 1) or non-accumulator of a specific element. The translocation capacity of an element, from roots to shoots, was evaluated by the translocation coefficient (TranslC = [total shoots element]/[total roots element]).

RESULTS AND DISCUSSION

Characteristics of the soils

Soils had great variability in their chemical characteristics, especially in the SD mining area. Soil characteristics (Tables 1 and 2) depend on materials where soils were developed. In fact, SD soils were developed on a very complex mixture of several mine wastes with or without host rocks, which contributes to a great heterogeneity in the soils properties.

The pH of the soils from SD varied between acid, for soils developed on schist and sediments, and influenced by acid mine drainage, and slightly alkaline in soils developed on mixtures of host rocks and gossan. The pH values in the mine area were lower than those in the CP area (Table 1). In both areas, electrical conductivities and N concentrations were small and C organic concentrations were small–medium. However, in one soil sample from SD was measured a high C organic concentration (125.2 g/kg), which was different compared to the values of the other samples.

Table 1 Chemical characteristics of rhizosphere soils of *Lavandula pedunculata* growing in São Domingos mining area and non-contaminated area – Corte do Pinto (min – max; mean). Values of the same row followed by an asterisk indicate significant differences between areas ($p < 0.05$).

	São Domingos mine (*n* = 6)	**Corte do Pinto (*n* = 3)**
pH (H_2O)	3.5 – 6.7	6.6 – 7.1
	4.7*	6.9*
EC (µS/cm)	57 – 383	104 – 423
	214	258
Organic C (g/kg)	9.4 – 125.2	14.3 – 22.5
	32.4	17.7
Total N (g/kg)	0.01 – 1.5	1.3 – 1.8
	0.9	1.5
Extractable P (mg/kg)	< 0.9 – 5.2	6.4 – 17.1
	3.7*	12.4*
Extractable K (mg/kg)	53.1 – 100.5	98.0 – 165.2
	66.3*	130.6*

EC: Electrical conductivity

Concentrations of extractable P and K were different between the areas (Table 1). Although concentrations of extractable P can be considered small in both areas (INIA – LQARS, 2000), soils from CP had 3-fold more extractable P than soils from SD. Soil extractable K concentrations were medium-high and very high to the SD and Corte do Pinto areas, respectively (INIA – LQARS, 2000). In general, soil fertility in Corte do Pinto was slightly higher, compared to the SD area.

The total concentrations of As, Fe, Pb, Sb, V and Zn were higher in soils from SD than from CP (Table 2), while the opposite was obtained for Cr and Mn. Despite of the variability, the total concentrations of Al, As, Cd, Ca, Cu, Fe, K, Mn, Ni, Pb, Sb, V and Zn in the soils from SD are in the

same range than those reported for mine wastes and/or soils developed on mine wastes from the same mine area (Freitas et al., 2004a, 2009; Pérez-López et al., 2008; Álvarez-Valero et al., 2008; Anawar et al., 2011, 2013; Abreu et al., 2012a,b; Santos et al., 2012, 2014; Durães et al., 2015).

For both areas, the total concentrations of As and Cu in the soils exceeded the maximum allowed values (MAV) for residential/parkland, commercial and industrial uses (12 mg As/kg and 63−91 mg Cu/kg; CCME, 2007). Moreover, all the studied soils from SD can be considered contaminated with Pb and Sb (160−600 mg Pb/kg and 20−40 mg Sb/kg, depending on land use; CCME, 2007), but only some samples are contaminated with V (MAV – 130 mg V/kg; CCME, 2007). The total concentrations of Cr and Ni in soils from CP were above MAV, but this fact is associated to the composition of the lithological materials (schists and greywackes) from which they were developed (Abreu et al., 2008; Tavares et al., 2008).

Significant differences were obtained between the concentrations of several elements in the soils available fraction (Table 3): the available concentrations of the elements associated to mining exploitation (As, Cd, Cu, Pb, Sb and Zn) were higher in soils from SD than in CP soils, while the opposite was observed for Ca, K, Mg and Mn.

Although soils from SD had higher concentrations of some elements in the total fraction, compared to the non-contaminated area (Table 2), the concentrations in the available fraction of the same elements in both areas were small (Table 3) corresponding to less than 5.8 % of the total. However in three soil samples from SD mine, Cd availability reached values corresponding to 16–29 % of the total concentration. For some nutrients (Cu, Fe, K, Mg, Mn, Mo, Ni and Zn), their availability was also small independently of the area, varying between 0.02 and 11 % of the total concentration, depending on the element. However, in SD, the soils developed on mixtures with considerable amounts of host rocks presented ≈20 % of the total Mn concentration in the available fraction. In general, Ca availability varied between 6 and 23 % of the total concentration in soils from all the studied areas (excepted one soil sample from SD that reached 49 %). The elements concentrations in the available fraction of the rhizosphere soils from mining areas depend on the extraction solution (Anjos et al., 2012; Santos et al., 2012). Additionally, the specific properties of the species can also determine significantly the availability of the elements for plants (Kabata-Pendias, 2004, 2011). Small concentrations of several studied elements (*e.g.* As, Ca, Cu, Fe, K, Mg, Mn, Ni, Pb, Sb and Zn) in the available fraction were also observed in the rhizosphere soils of other species (*e.g. Cistus* and *Erica* genus) growing in SD mine (Abreu et al., 2008, 2012a,b; Santos et al., 2012, 2014; Perez-Lopez et al., 2014; Durães et al., 2015). Even in the mine wastes from which some of the studied soils were developed, the available fractions of elements are usually small (Pérez-López et al., 2008).

In general, the chemical properties of soils from SD, like pH and total concentrations of organic C and other studied elements, did not explain the small elements concentrations in the available fraction. Only for Zn, a positive correlation (r = 0.95) between total and the available fraction concentrations was found. In fact, the low availability of As, Cu, Pb, Mn and Zn in soils from SD is mainly related to: the association of these elements mostly to the residual fraction (in the crystalline structure of silicates or other solid phases as salts with low solubility) and Fe−oxides fraction, (Abreu et al., 2012a; Santos et al., 2012; Durães et al., 2015).

Table 2 Total concentrations of the chemical elements in the rhizosphere soils of *Lavandula pedunculata* growing in São Domingos mining area and non-contaminated area – Corte do Pinto (min – max; mean).

	São Domingos mine (*n* = 6)	**Corte do Pinto** (*n* = 3)
	Potentially hazardous elements (mg/kg)	
Al	$34.4 \times 10^3 – 83.0 \times 10^3$	$66.0 \times 10^3 – 93.5 \times 10^3$
	64.6×10^3	82.8×10^3
As	871 – 3180	38.4 – 79.2
	1702*	57.0*
Cd	< 0.3 – 1.4	< 0.3
	0.6	
Cr	75 – 93	116 – 157
	84*	133*
Pb	425 – 5300	38 – 105
	3039*	62*
Sb	73 – 249	6.9 – 10.6
	203*	8.9*
V	91 – 183	66 – 87
	130*	78*
	Nutrients (mg/kg)	
Ca	$0.6 \times 10^3 – 3.2 \times 10^3$	$3.1 \times 10^3 – 32.9 \times 10^3$
	1.5×10^3*	13.6×10^3*
Cu	67 – 1310	48 – 229
	543	123
Fe	$52.2 \times 10^3 – 118.0 \times 10^3$	$43.1 \times 10^3 – 52.9 \times 10^3$
	90.4×10^3*	49.2×10^3*
K	$7.4 \times 10^3 – 19.2 \times 10^3$	$5.4 \times 10^3 – 10.5 \times 10^3$
	16.3×10^3	7.9×10^3
Mg	800 – 5900	4300 – 5900
	3000	5300
Mn	91 – 504	1450 – 1980
	277*	1663*
Mo	<1 – 10	2 – 4
	3	3
Ni	18 – 70	54 – 75
	33	65
Zn	80 – 857	61 – 79
	295*	71*

Values of the same row followed by an asterisk indicate significant differences between areas ($p < 0.05$).

Table 3 Concentrations of the chemical elements in the available fraction of the rhizosphere soils of *Lavandula pedunculata* growing in São Domingos mining area and non-contaminated area – Corte do Pinto (min – max; mean).

	São Domingos mine (*n* = 6)	**Corte do Pinto (*n* = 3)**
	Potentially hazardous elements (mg/kg)	
Al	113.9 – 315.8	75.1 – 150.1
	169.3	118.1
As	0.2 – 6.0	0.03 – 0.3
	2.0*	0.1*
Cd	0.01 – 0.06	0.003 – 0.005
	0.04*	0.004*
Cr	0.03 – 0.3	0.2 – 0.3
	0.08	235.7
Pb	0.1 – 0.4	0.01 – 0.1
	0.3*	0.04*
Sb	0.1 – 0.6	<0.001 – 0.02
	0.3*	0.01*
V	0.02 – 0.1	<0.01 – 0.05
	0.07	0.03
	Nutrients (mg/kg)	
Ca	57.0 – 781.2	558.5 – >1982
	278.5*	1215*[1]
Cu	0.9 – 22.1	0.3 – 0.6
	8.0*	0.5*
Fe	135.6 – 489.2	84.1 – 239.6
	245.8	187.4
K	30.1 – 45.3	50.2 – 91.8
	38.1*	67.0*
Mg	21.5 – 94.0	197.6 – 269.6
	47.0*	227.6*
Mn	4.4 – 106.6	83.3 – 206.4
	27.7*	150.2*
Mo	0.002 – 0.04	<0.001 – 0.04
	0.01	0.03
Ni	0.1 – 0.6	0.2 – 0.4
	0.3	0.3
Zn	3.0 – 83.7	0.7 – 3.0
	19.9*	2.0*

[1]Estimated mean considering the over range value.
Values of the same row followed by an asterisk indicate significant differences between areas ($p < 0.05$).

Even in the mine wastes from which some of the studied soils were developed, several elements (*e.g.* Cd, Cr, Fe, Sb, among others) are mainly associated to the residual fraction (Pérez-López et al., 2008). However, in soils from CP, the organic C concentrations can lead to the decrease of the availability of several cations as Cd, Fe, Mn, Ni and V ($-0.96 < r < -1.00$, depending on the element). The increase of the total concentrations of Al, As, Ca, Fe, Ni, Pb and Sb led to the high concentrations of the same elements in the soils available fraction ($0.91 < r < 1.00$, depending on element).

Although soil characteristics as low fertility and pH, as well as the multielemental contamination can be considered strong limiting factors for plant growth, *L. pedunculata* is able to survive in soils with quite different properties, including different levels of contamination, showing a reasonable vegetative development.

Chemical composition of Lavandula pedunculata

Concentrations of the potentially hazardous elements (Table 4) and nutrients (Table 5) in plant roots from SD presented great variability being always higher than the available concentrations of the same elements in the respective soils. No correlation was obtained between elements concentrations in the roots and the soil available fraction, except for Al and Cu. For these cations, the increasing of their available concentrations contributed to the highest concentrations of the same elements in the roots ($r_{Al} = 0.98$; $r_{Cu} = 0.96$).

In SD, an intrapopulation variation in the translocation of some elements from roots to shoots was observed. The majority of the nutrients (Ca, Fe, Mn, Mo, Ni and Zn) were mainly translocated from roots to shoots due to their important metabolic functions (TranslC: 1.0–6.3, depending on the element), as was also observed for other species growing in the same mine area (Freitas et al., 2009; Abreu et al., 2012a,b; Pérez-López et al., 2014; Santos et al., 2014; Durães et al., 2015). Arsenic, Cr, Sb and V were also translocated from roots to shoots (TranslC: 1.0−5.0, depending on the element), but the concentrations of these potentially hazardous elements (except As) in the shoots (Table 4) were considered normal/sufficient or below the phytotoxic level (Kabata-Pendias, 2011).

The other studied potentially hazardous elements (Al, Cd and Pb) were mainly stored in the roots (TranslC < 1.0). The storage of the potentially hazardous elements in the roots and their decrease in the aerial parts of the plants is considered an efficient tolerance mechanism under metal/metalloid stress (Kabata-Pendias, 2011), which has also been reported for several species growing in the same mine area (Freitas et al., 2009; Abreu et al., 2012a,b; Santos et al., 2012, 2014; Pérez-López et al., 2014; Durães et al., 2015).

Low translocation of Cu (TranslC < 1) was also observed, but the concentrations of this element in the shoots were considered normal and sufficient according to the values referred for plants in general (Kabata-Pendias, 2011). Copper retention in *L. pedunculata* roots growing in soils with high total concentrations of this element, as is the case of soils from SD mine area, can be a tolerance mechanism that guarantees adequate levels of Cu in the photosynthetic parts of this species. In fact, the first tolerance mechanism in the plants seems to be the avoidance of accumulation of toxic concentrations of the elements in sensitive plant parts, preventing their damage effects, rather than

triggering other cellular mechanism of tolerance/detoxification (Yruela, 2005). In SD mine, the Cu storage in plant roots is also reported for *E. australis* and *E. andevalensis* (Pérez-López et al., 2014) as well as in some plants of *Cistus* species (Abreu et al., 2012a,b; Santos et al., 2012, 2014).

Table 4 Concentrations of the potentially hazardous elements in the roots and shoots of *Lavandula pedunculata* collected in São Domingos mining area and non-contaminated area – Corte do Pinto (min – max; mean).

(mg/kg)	São Domingos mine		Corte do Pinto	References
	Roots (*n* = 6)	**Shoots (*n* = 6)**	**Shoots (*n* = 3)**	
Al	322.8 – 1465 726.1	398.1 – 612.2 499.7	383.1 – 506.2 460.7	—
As	4.1 − 23.9 12.3	3.2 – 26.9 14.6*	0.3 – 3.8 1.6*	5.05 ± 0.36[1] 7.73 ± 1.75[1] 14.2[2] 1.67[4a] 11.2[4b] 3.60[4c]
Cd	0.3 – 1.2 0.8	0.3 – 2.3 0.8*	0.04 – 0.1 0.05*	0.5 – 1.2[5] 0.6[3]
Cr	1.6 – 2.8 2.1	1.8 – 3.4 2.4	2.1 – 2.8 2.4	8.56 ± 2.09[1] 12.2 ± 0.26[1] 6.6[2] 1.5 – 29.5[6]
Pb	11.0 – 52.7 26.3	5.9 – 53.0 21.7*	0.7 – 8.9 3.7*	6.96[4a] 9.21[4b] 3.08[4c] 11.9 – 19.5[5] 1.1 – 12.1[6]
Sb	0.05 – 0.7 0.2	0.2 – 1.2 0.5*	0.03 – 0.2 0.1*	0.52 ± 0.27[1] 1.83 ± 0.64[1] 1.9[2]
V	0.4 – 0.9 0.6	0.6 – 1.1 0.9	0.6 – 0.8 0.7	—

[1]Anawar et al. (2011), leaves of *L. luisieri* L. collected in different areas from São Domingos mine; [2]Anawar et al. (2013), shoots of *L. stoechas* L. collected in São Domingos mine; [3]Boularbah et al. (2006), shoots of *L. dentata* L. from mining sites in South Morocco; [4a]de la Fuente et al. (2010), aerial part of *L. stoechas* subsp. *luisier*i (Rozeira) Rozeira from Rio Tinto mining region; [4b]de la Fuente et al. (2010), aerial part of *L. viridis* L'Her from Rio Tinto mining region; [4c]de la Fuente et al. (2010), aerial part of *L. sampaioana* (Rozeira) Rivas Mart., T.E. Díaz & Fern. Gonz. from Rio Tinto mining region; [5]Freitas et al. (2004a), leaves and twigs of *L stoechas* L. subsp. *pedunculata* Samp. and Rozeira from São Domingos mine area; [6]Freitas et al. (2004b), leaves, twigs and aerial part of *L. stoechas* L. subsp. *sampaiana* Rozeira from abandoned mining area of Pingarela
Values of the same element in the shoots followed by an asterisk indicate significant differences between populations ($p < 0.05$).

Interpopulation variation was observed for the concentrations of As, Cd, Sb, Pb and Zn in the shoots (Table 4). Thus, concentrations of these elements in this organ from SD plants were higher than those collected in the non-contaminated area. For the other studied elements (Tables 4 and 5), no significant differences were obtained in spite of some plant samples collected in SD area had reached high elements concentrations (*e.g.* Cu, Fe, Mn, Mo).

Table 5 Concentrations of the nutrients in the roots and shoots of *Lavandula pedunculata* collected in São Domingos mining area and non-contaminated area – Corte do Pinto (min – max; mean).

(mg/kg)	São Domingos mine		Corte do Pinto	References
	Roots (*n* = 6)	**Shoots (*n* = 6)**	**Shoots (*n* = 3)**	
Ca	$2.3 \times 10^3 – 2.8 \times 10^3$ 2.5×10^3	$5.7 \times 10^3 – 10.1 \times 10^3$ 8.3×10^3	$7.8 \times 10^3 – 8.9 \times 10^3$ 8.2×10^3	(8.9×10^3)[4a] (4.8×10^3)[4b] (9.1×10^3)[4c] 125[3]
Cu	12.3 – 251 70.2	7.5 – 30.8 18.0	7.7 – 11.4 8.9	49.3[4a] 39.7[4b] 45.2[4c] 5.5 – 8.5[5] 5.2 – 46.8[6]
Fe	390 – 1120 660	440.0 – 1820 980	310 – 570 440	301 ± 7[1] 633 ± 33[1] 1393[2] 454[4a] 643[4b] 474[4c]
K	>310	>730	>730	283.2 – 3139.1[6] 11620[2]
Mg	> 310	>730	> 730	—
Mn	69.5 – 231.7 130.3	136.0 – 685.1 427.3	295.0 – 408.0 355.4	1291[4a] 2208[4b] 2391[4c] 16.4 – 187.1[6]
Mo	0.05 – 0.2 0.1	0.1 – 0.7 0.3	0.1 – 0.2 0.1	—
Ni	1.9 – 4.1 2.8	1.7 – 5.4 3.7	2.6 – 3.5 3.0	2.36[4a] 8.96[4b] 3.32[4c] 1.2 – 2.7[5] 10.7 – 118.4[6]
Zn	63.7 – 129.2 106.0	183.3 – 345.1 229.0*	27.4 – 47.4 34.3*	102 ± 10[1] 325 ± 3[1] 134[2] 174[3] 139[4a] 301[4b] 188[4c] 76.9 – 201.7[5] 16.0 – 137.4[6]

[1]Anawar et al. (2011), leaves of *L. luisieri* L. collected in different areas from São Domingos mine; [2]Anawar et al. (2013), shoots of *L. stoechas* L. collected in São Domingos mine; [3]Boularbah et al. (2006), shoots of *L. dentata* L. from mining sites in South Morocco; [4a]de la Fuente et al. (2010), aerial part of *L. stoechas* subsp. *luisier*i (Rozeira) Rozeira from Rio Tinto mining region; [4b]de la Fuente et al. (2010), aerial part of *L. viridis* L'Her from Rio Tinto mining region; [4c]de la Fuente et al. (2010), aerial part of *L. sampaioana* (Rozeira) Rivas Mart., T.E. Díaz & Fern. Gonz. from Rio Tinto mining region; [5]Freitas et al. (2004a), leaves and twigs of *L stoechas* L. subsp. *pedunculata* Samp. and Rozeira from São Domingos mine area; [6]Freitas et al. (2004b), leaves, twigs and aerial part of *L. stoechas* L. subsp. *sampaiana* Rozeira from abandoned mining area of Pingarela.
Values of the same element in the shoots followed by an asterisk indicate significant differences between populations ($p < 0.05$).

Considering the two populations as a cluster, the elements concentrations in the shoots were not correlated to the concentrations of the same elements in the soils (total and available fractions). However, considering the independent populations adapted to their specific environments, the concentrations of Al, Ca and V in the plant shoots from CP were correlated to the concentrations of the same elements in the available fraction of the soils (r = 0.90−0.98, depending on the element). The same was not observed for the population from SD for any of the studied elements. In fact, the different concentrations of the elements in the shoots are the combined result of uptake, accumulation in roots, translocation from roots to shoots and tolerance capacity (Kabata-Pendias, 2011). Nonetheless, independently of the population, all plants showed higher concentrations of some elements in shoots than in the soil available fraction.

For most of the plants from both areas, shoots had concentrations of Cd, Cr, Cu, Mo, Ni, Sb and V as well as Fe and Pb (except in two samples from SD, which exceeded phytotoxic levels) below the range considered toxic and/or within the normal/sufficient range for plants (Kabata-Pendias, 2011). Plant shoots from CP also had concentrations of Mn and Zn considered normal/sufficient and lower than phytotoxic (Kabata-Pendias, 2011).

The concentrations of As, Mn (except in two samples) and Zn in the plant shoots from SD exceeded the phytotoxic values reported by Kabata-Pendias (2011) for most plant species, but visual signs of toxicity were not observed. Phytotoxic concentrations of Zn and Mn in the shoots seem to have a different effect in the concentrations of Cd (r = 0.94) and Pb (r = −0.93), respectively, in the same organ.

Plant parts (shoots, leaves or twigs) of different *Lavandula* species collected in SD mine or other mining areas under arid and semi-arid climate conditions showed a wide range of concentrations for some of the studied elements (Tables 4 and 5). In some cases, similar values compared to those obtained in the present study were reported by several authors (Freitas et al., 2004a,b; Boularbah et al., 2006; de la Fuente et al., 2010; Anawar et al., 2011, 2013).

For the same species, *L. pedunculata*, different concentrations of several elements (As, Ca, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in shoots were reported for plants growing in Rio Tinto mining region (de la Fuente et al., 2010) and in a serpentine area from north–east of Portugal (old mining area of Pingarela), where the climatic conditions are different (Freitas et al., 2004b). However, according to the results reported by the same authors, some plant samples have some elements concentrations (As, Ca, Cr, Cu, Fe, Ni, Pb and Zn) in the same range than those obtained in this study. These differences can be explained by seasonal and temporal variations in plants and soils as well as the analysed plant part. Moreover, interpopulation variability can exist as reported for other species growing in mining areas (Abreu et al., 2012a; Santos et al., 2012, 2014).

The calculated values of the soil–plant transfer coefficient indicated that the two populations are accumulators of Ca (TransfC > 1) and non-accumulators of the other studied elements (TransfC < 1). Intrapopulation variation in the accumulation behaviour of Cd, Mn and Zn was observed in SD being some plants accumulators of these elements (TransfC − Cd: 0.1−15.1; Mn: 0.3−4.8; Zn: 0.2−4.3). Considering the calculated values of the soil to plant transfer coefficients, no general pattern of elements accumulation was linked to the essential nutritional requirements for plant species.

Lavandula pedunculata from Rio Tinto mining region was also accumulator of Mn and Zn and non-accumulator of As, Fe, Ni and Pb. However, an opposite accumulator behaviour, compared to the present study, was observed for Ca and Cu (de la Fuente et al., 2010). In plants of *Lavandula* genus growing in other contaminated areas (Boularbah et al., 2006; de la Fuente et al., 2010), the accumulator behaviour, indicated by the same coefficient, depended on species and elements being, in some cases, similar to that obtained in this study.

Physiological characterization of Lavandula pedunculata

Some species or ecotypes, like *L. pedunculata* growing in contaminated soils from SD mining area, did not show any visual symptoms of toxicity even with elements concentrations in their shoots considered as phytotoxic (As, Mn and Zn). However, at the physiological level, several phytotoxic effects and consequent responses can occur.

In general, the excess of potentially toxic elements in shoots can modify the pigments concentrations, which are usually linked to visual symptoms of plant disease and photosynthetic activity (Pang et al., 2003; Stoeva and Bineva, 2003). Although no visual alteration in the shoots colour was observed in plants collected both in SD mining area and in non-contaminated area (CP), significant differences were obtained between chlorophylls concentrations (Fig. 1) in the plants from the two areas. However, the same was not observed for the carotenoids concentrations (Fig. 1).

Concentrations of Chl*a* in the shoots were similar for the two populations, while Chl*b* in the shoots from SD reached the highest values. This fact contributed to the significant differences of the total chlorophylls concentration in the plant shoots from both populations. Although plant shoots collected in CP had elements concentrations considered normal and/or below phytotoxicity, a negative effect of Al, Fe, Ni and V on total chlorophylls was observed ($-0.93 < r < -0.99$, depending on the element), possibly due to the low tolerance of this population to those chemical elements. Moreover, Al concentrations in plant shoots from CP also seem to negatively affect the Chl*b* concentration ($r = -1.00$). The decrease of the chlorophyll content in the shoots from plants collected in the non-contaminated area can be related to the inhibition of the synthesis of photosynthetic pigments and/or enzymes responsible for chlorophyll biosynthesis as well as chlorophyll degradation by the increase of chlorophyllase activity (Sharma and Dubey, 2005).

The chlorophyll ratio, used as a stress indicator (Zengin and Munzuroglu, 2005), was high in the plant shoots from CP (SD: 1.2−1.7; CP: 1.7−1.9) suggesting the existence of plant stress. In fact, the increase of the chlorophyll ratio due to oxidative stress has been reported in *Phaseolus vulgaris* L. with the application of increasing doses of Cd, Cu and Pb in controlled conditions (Zengin and Munzuroglu, 2005).

Protein contents in plants are also affected by the high concentrations of potentially toxic elements in plants, as a result of the reduction of its biosynthesis or the acceleration of its degradation (Stoeva and Bineva, 2003). However, in the present study, similar concentrations of total protein were obtained in the two *L. pedunculata* populations (Table 6).

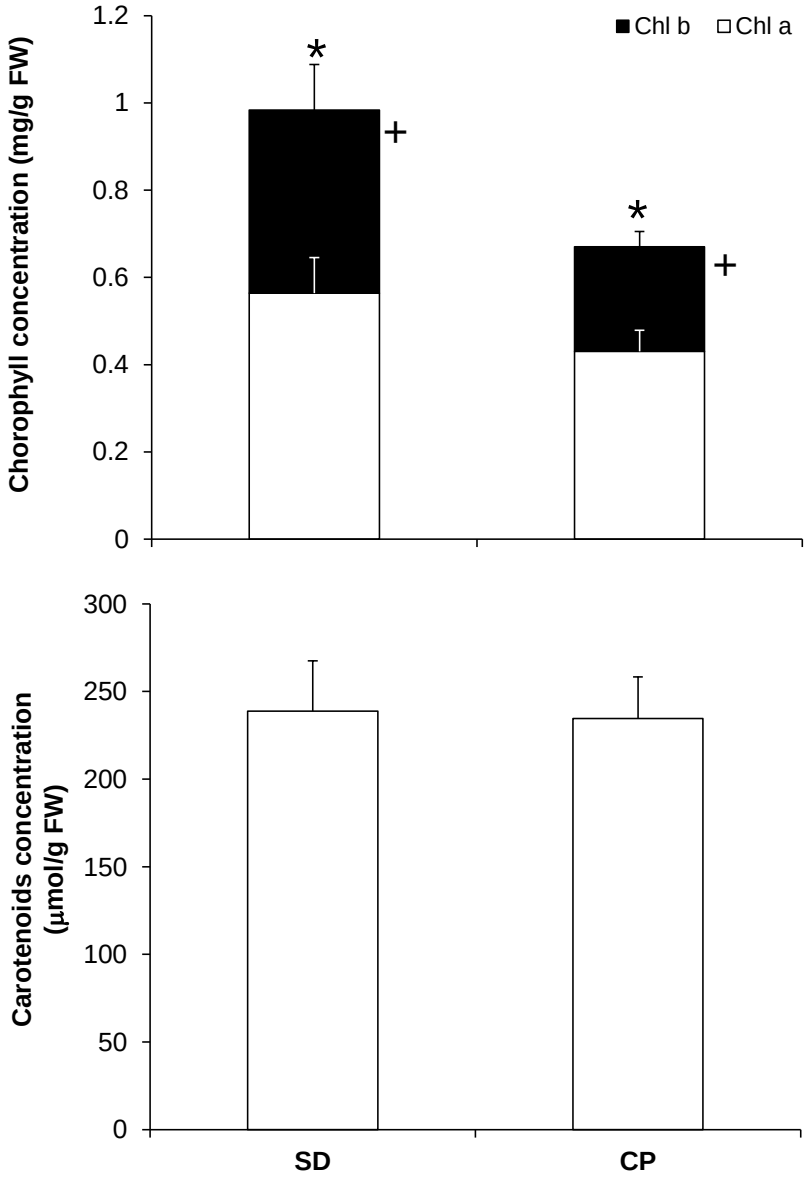

Fig. 1 Concentration of chlorophyll (total, *a* and *b*) and carotenoids in *Lavandula pedunculata* shoots collected in São Domingos mine area (SD) and non-contaminated area – Corte do Pinto (CP) (mean ± SD; *n* = 6 and 3, respectively).Values followed by the following symbols indicate significant differences between the populations ($p < 0.05$): total chlorophyll (*), chlorophyll *a* (++), chlorophyll *b* (+) and carotenoids (•).

Under oxidative stress, plants can increase the activities of non-enzymatic components as response to the scavenging of ROS (Pang et al., 2003; Cao et al., 2004). Concerning the non-enzymatic components (Table 6), no significant differences were obtained between concentrations of proline and acid-soluble thiols in the shoots collected in both areas. Similar proline contents were also obtained in *Cardaminopsis arenosa* (L.) Hayek leaves growing in some metalliferous and non-contaminated areas, both from southern of Poland (Nadgórska-Socha et al., 2013). However, according to the same authors, *Plantago lanceolata* L. leaves from the same contaminated area had lower proline concentrations than those from the non-contaminated area. Kandziora-Ciupa et al. (2013) also reported no significant effect of heavy metals in the accumulation of proline in *Vaccinium*

myrtillus L. leaves. Nonetheless, proline accumulation was observed in leaves of *V. zizanioides* growing in tailings with different proportions of Pb/Zn (Pang et al., 2003).

Table 6 Physiological parameters in *Lavandula pedunculata* shoots collected in São Domingos mining area and non-contaminated area – Corte do Pinto (min – max; mean).

	São Domingos mine (*n* = 6)	**Corte do Pinto (*n* = 3)**
Total protein (mg/g)	3.7 – 8.7 6.1	7.2 – 10.4 8.6
Non-enzymatic components		
Acid soluble thiols (µmol SH/g)	0.16 – 0.19 0.18	0.19 – 0.21 0.20
Proline (µg/g)	101.1 – 157.3 128.9	103.8 – 129.5 117.6
Enzymatic components		
CAT activity (µmol H_2O_2 min^{-1} g^{-1})	148.7 – 239.9 187.0*	257.2 – 350.6 289.4*
Specific CAT activity (µmol H_2O_2 min^{-1} mg^{-1} protein)	23.4 – 40.0 32.2	31.1 – 36.0 33.6
SOD activity (U/g)	76.7 – 159.2 131.3	48.4 – 202.6 127.7
Specific SOD activity (U/mg protein)	15.3 – 29.3 22.4	5.8 – 19.5 14.6

Values of the same row followed by an asterisk indicate significant differences between populations ($p < 0.05$).

Differences of thiols concentrations in several plant species collected in contaminated and non-contaminated areas were reported by Kandziora-Ciupa et al. (2013) and Nadgórska-Socha et al. (2013), which are not in agreement with the findings in the present study (Table 6). Comparing both contaminated and non-contaminated areas, it was noticed by Kandziora-Ciupa et al. (2013) an increase of the thiols concentrations in the leaves of *V. myrtillus* growing in the contaminated area.

Under oxidative stress, plants can also produce or stimulate the activity of antioxidative enzymes that remove and neutralize ROS (Pang et al., 2003; Mishra et al., 2006). In the shoot samples of *L. pedunculata*, the activities of SOD and CAT followed different patterns (Table 6). Independently of the plant population, no variation was observed in the total and specific SOD activities in the shoots. A similar result was reported by Santos et al. (2009) for mature leaves of *C. ladanifer* also collected in SD mine and Pomarão (non-contaminated area near the mine area) and same sampling period (Spring). Superoxide dismutase activities in *C. arenosa*, *P. lanceolata* and *Viola tricolor* L. depend on population, however in some cases similar enzymatic activities were also obtained for plants collected in contaminated and non-contaminated sites (Słomka et al., 2008; Nadgórska-Socha et al., 2013).

The levels of the total activity of CAT enzyme varied with plant population (Table 6) attaining the lowest values in the shoots from SD plants. However, the specific CAT activity was similar owing to the high protein content in the plants from CP. This indicates that CAT synthesis did not follow total protein synthesis. The lower total activity of CAT in the shoots from SD was not correlated to the phytotoxic concentrations of As, Mn and Zn in the same organ, or even the concentrations of other studied elements. In natural conditions, non-specific stress responses of *L. pedunculata* from SD can be explained by the co-existence of different stress factors like high concentrations of several elements in the shoots, water deficiency and high intensity of UV radiation.

The total activity of CAT in the shoots from CP was affected by Mn concentrations in the same organ (r = −0.93). Although concentration of this metal in the shoots is considered sufficient and below phytotoxicity, the tolerance of this population can be different compared to SD population. In general, the activity of antioxidative enzymes only increases until tolerable elements contents and, when elements concentrations exceed this level a subsequent decrease of enzymes activity occurs (Cao et al., 2004).

Low CAT activity was also observed in young leaves of *C. ladanifer* collected in SD mine, compared to leaves from a non-contaminated area (Santos et al., 2009). On the contrary, CAT activity in *V. tricolor* leaves from contaminated heaps was higher than in control (non-contaminated area) (Słomka et al., 2008). The increase of CAT activity was also observed in leaves of *V. zizanioides* growing in tailings with different proportions of Pb/Zn (Pang et al., 2003).

The response of antioxidant mechanisms to phytotoxic concentrations of elements depends on the level of free radicals resulting from the balance between their production and scavenging. So, *L. pedunculata* growing in multielemental contaminated soils accumulate phytotoxic concentrations of As, Mn and Zn in shoots but these elements seem to be efficiently compartmentalized into cellular parts where it is not necessary to trigger an antioxidative response. Additionally, populations from SD mining area can have higher tolerance range to potentially hazardous elements.

CONCLUSIONS

In São Domingos mining area, the soils developed on different mine wastes showed a great heterogeneity of their chemical characteristics. Independently of the substrata, *Lavandula pedunculata* is able to grow in soils containing different levels of multielemental contamination, low fertility and a wide range of pH. Nonetheless, the plants did not show any visible symptoms of phytotoxicity and/or nutritional deficiency.

Lavandula pedunculata showed inter- and intrapopulation variation in the concentrations of the studied elements in the shoots. Populations of São Domingos and Corte do Pinto (non-contaminated area) were accumulators of Ca and non-accumulators of the other studied elements. But some intrapopulation variation in the accumulation behaviour of Cd, Mn and Zn was observed in São Domingos plants.

The tolerance of São Domingos population to potentially hazardous elements seem to be firstly related to elements storage in roots, preventing their translocation to the photosynthetic tissues, rather

than intracellular production of non-enzymatic compounds and activity of antioxidative enzymes. In fact, phytotoxic concentrations of As, Mn and Zn in the shoots from São Domingos plants did not affect the concentrations of pigments and total protein, and trigger antioxidative mechanisms by enzymatic and non-enzymatic components. The lowest tolerance of Corte do Pinto population, compared to São Domingos, can also be noticed by the reduction of the total chlorophylls and Chlb concentrations even in the presence of concentrations of some elements (*e.g.* Al, Fe, Ni and V) in the shoots below phytotoxic level.

Overall, these results reveal that *L. pedunculata* has potential for phytostabilisation of soils with mutielemental contamination under Mediterranean conditions. However, to ensure a similar ecological behaviour of the plants and their tolerance to the co-existence of several stress factors, the use of seeds collected in the mining areas in phytostabilisation programs is recommended.

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REFERENCES

Abreu, M.M., Magalhães, M.C.F., 2009. Phytostabilization of soils in mining areas. Case studies from Portugal. In: Aachen, L., Eichmann, P. (Eds.), Soil remediation. Nova Science Publishers, Inc. New York, pp. 297-344.

Abreu, M.M., Tavares, M.T., Batista, M.J., 2008. Potential use of *Erica andevalensis* and *Erica australis* in phytoremediation of sulphide mine environments: São Domingos, Portugal. Journal of Geochemical Exploration 96(2-3), 210-222.

Abreu, M.M., Santos, E.S., Ferreira, M., Magalhães, M.C.F., 2012a. *Cistus salviifolius* a promising species for mine wastes remediation. Journal of Geochemical Exploration 113, 86-93.

Abreu, M.M., Santos, E.S., Fernandes, E., Magalhães, M.C.F., 2012b. Trace elements tolerance, accumulation and translocation in *Cistus populifolius*, *Cistus salviifolius* and their hybrid growing in polymetallic contaminated mine areas. Journal of Geochemical Exploration 123, 52-60.

Álvarez-Valero, A.M., Pérez-López, R., Matos, J., Capitán, M.A., Nieto, J.M., Sáez, R., Delgado, J., Caraballo, M., 2008. Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian Peninsula): evidence from a chemical and mineralogical characterization. Environmental Geology 55(8), 1797-1809.

Activation Laboratories, 2015a Code 1H – Au + 48. Available in: http://www.actlabs.com/page.aspx?page=506&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes. Accessed May 2015.

Activation Laboratories, 2015b. Code 6 – Hydrogeochemistry – ICP/MS. Available in: http://www.actlabs.com/page.aspx?page=544&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes. Accessed May 2015.

Activation Laboratories, 2015c. 2D - Vegetation Ash - ICP/MS. Available in: http://www.actlabs.com/page.aspx?page=538&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes. Accessed May 2015.

Anawar, H.M., Freitas, M.C., Canha, N., Regina, I.S., 2011. Arsenic, antimony, and other trace element contamination in a mine tailings affected area and uptake by tolerant plant species. Environmental Geochemistry and Health 33, 353-362.

Anawar, H.M., Canha, N., Santa-Regina, I., Freitas, M.C. 2013. Adaptation, tolerance, and evolution of plant speciesin a pyrite mine in response to contamination leveland properties of mine tailings: sustainable rehabilitation. Journal of Soils and Sediments 13, 730-741.

Anjos, C., Magalhães, M.C.F., Abreu, M.M., 2012. Metal (Al, Mn, Pb and Zn) soils extractable reagents for available fraction assessment: Comparison using plants, and dry and moist soils from the Braçal abandoned leadmine area, Portugal. Journal of Geochemical Exploration 113, 45-55.

Bates, L., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant and Soil 39, 205-207.

Boularbah, A., Schwartz, C., Bitton, G., Aboudrar, W., Ouhammou, A., Morel, J.L., 2006. Heavy metal contamination from mining sites in South Morocco: 2. Assessment of metal accumulation and toxicity in plants. Chemosphere 63, 811-817.

Bradford, M.M., 1976. A Rapid and sensitive method for the quantitation ofmicrogram quantities of protein utilizing theprinciple of protein-dye binding. Analytical Biochemistry 72, 248-254.

Campostrini, E., Glenn, D., 2007. Ecophysiology of papaya: A review. Brazilian Journal of Plant Physiology 19(4), 413-424.

Cao, X., Ma, L.Q., Tu, C., 2004.Antioxidative responses to arsenic in the arsenic-hyperaccumulator chinese brake fern (*Pteris vittata* L.). Environmental Pollution 128, 317-325.

CCME – Canada Council of Ministers of the Environment, 2007. Canadian soil quality guidelines for the protection of environmental and human health: Summary tables (updated September, 2007). CCME, Winnipeg, Canada.

Chance, B., Maehly, A.C., 1955. Assay of catalases and peroxidases. Methods in Enzymology 2, 764-817.

de la Fuente, V., Rufo, L., Rodríguez, N., Amils, R., Zuluaga, J., 2010.Metal accumulation screening of the Río Tinto Flora (Huelva, Spain). Biological Trace Element Research 134, 318-341.

Durães, N., Bobos, I., Ferreira da Silva, E., Dekayir, A., 2015.Copper, zinc and lead biogeochemistry in aquatic and land plants from the Iberian Pyrite Belt (Portugal) and north of Morocco mining areas. Environmental Science and Pollution Research 22, 2087-2105.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 82, 70-77.

Feng, M.H., Shan, X.Q., Zhang, S.Z., Wen, B., 2005. A comparison of the rhizosphere-based method with DTPA, EDTA, $CaCl_2$, and $NaNO_3$ extraction methods for prediction of bioavailability of metals in soil to barley. Environmental Pollution 137, 231-240.

Freitas, H., Prasad, M.N.V., Pratas, J., 2004a. Plant community tolerant to trace elements growing on degraded soils of São Domingos mine in the south east of Portugal: environmental implications. Environment International 30, 65-72.

Freitas, H., Prasad, M.N.V., Pratas, J., 2004b. Analysis of serpentinophytes from north–east of Portugal for trace metal accumulation − relevance to the management of mine environment. Chemosphere 54, 1625-1642.

Freitas, M.C., Pacheco, A.M.G., Anawar, H.M., Dionísio, I., Dung, H.M., Canha, N., Bettencourt, A., Henriques, F., Pinto-Gomes, C.J., Capelo, S., 2009. Determination of phytoextraction potential of plant species for toxic elements in soils of abandoned sulphide-mining areas. Journal of Radioanalytical and Nuclear Chemistry 282, 21-27.

Grant, J.J., Loake, G.J., 2000. Role of active oxygen intermediates and cognate redox signalling in disease resistance. Plant Physiololy 124, 21-29.

INIA–LQARS, 2000. Manual de fertilização das culturas. Laboratório Químico Agrícola Rebelo da Silva (Ed.), Lisboa.

IUSS Working Group WRB, 2007. World reference base for soil resources 2006 (first update 2007). World Soil Resources Reports No. 103, Rome, FAO.

Kabata-Pendias, A., 2004. Soil-plant transfer of trace elements-an environmental issue. Geoderma 122, 143-149.

Kabata-Pendias, A., 2011. Trace elements in soils and plants. CRC Press, Boca Raton.

Kandziora-Ciupa, M., Ciepał, R., Nadgórska-Socha, A., Barczyk, G., 2013. A comparative study of heavy metal accumulation and antioxidant responses in *Vaccinium myrtillus* L. leaves in polluted and non-polluted areas Environmental Science and Pollution Research 20, 4920-4932.

Kirk, J.T.O., Allen, R.L., 1965. Dependence of chloroplast pigment synthesis on protein synthesis: effects of actidone. Biochemical and Biophysical Research Communications 21, 523-530.

Khopde, S.M., Priyadarsini, K.I., Mohan, H., Gawandi, V.B., Satav, J.G., Yakhmi, J.V., Banavaliker, M.M., Biyani, M.K., Mittal, J.P., 2001. Characterizing the antioxidant activity of amla (*Phyllanthus emblica*) extract. Current Science 81, 185-190.

Mackinney, G., 1941. Absortion of light by chlorophyll solutions. The Journal of Biological Chemistry 144, 315-323.

Matos, J.X., Martins, L.P., 2006. Reabilitação ambiental de áreas mineiras do sector português da Faixa Piritosa Ibérica: estado da arte e perspectivas futuras. Boletin Geológico y Minero 117, 289-304.

Mishra, S., Srivastava, S., Tripathi, R.D., Kumar, R., Seth, C.S., Gupta, D.K., 2006. Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere 65, 1027-1039.

Nadgórska-Socha, A., Ptasiński, B., Kita, A., 2013. Heavy metal bioaccumulation and antioxidative responses in *Cardaminopsis arenosa* and *Plantago lanceolata* leaves from metalliferous and non-metalliferous sites: a field study. Ecotoxicology 22, 1422-1434.

Pang, J., Chan, G.S.Y., Zhang, J., Liang, J., Wong, M.H., 2003. Physiological aspects of vetiver grass for rehabilitation in abandoned metalliferous mine wastes Chemosphere 52, 1559-1570.

Pérez-López, R., Nieto, J.M., Almodóvar, G.R., 2007. Immobilization of toxic elements in mine residues derived from mining activities in the Iberian Pyrite Belt (SW Spain): Laboratory experiments. Applied Geochemistry. 22, 1919-1935.

Pérez-López, R., Márquez-García, B., Abreu, M.M., Nieto, J.M., Córdoba, F., 2014. *Erica andevalensis* and *Erica australis* growing in thesame extreme environments: Phytostabilization potential of mining areas. Geoderma 230-231, 194-203.

Póvoas, I., Barral, M.F., 1992. Métodos de análise de solos. Comunicações do Instituto de Investigação Científica Tropical. Serie Ciências Agrárias 10, Lisboa.

Quental, L., Bourguignon, A., Sousa, A.J., Batista, M.J., Brito, M.G., Tavares, T., Abreu, M.M., Vairinho, M., Cottard, F., 2002. MINEO Southern Europeenvironment test site. Contamination impact mapping and modelling – Final Report. Assessing and monitoring the environmental impact of mining activities in Europe using advanced Earth Observation Techniques (MINEO) 5yth FP-IST-1999-10337.

Riddles, P.W., Blakeley, R.L., Zerner, B., 1979. Ellman's reagent: 5, 5´-dithiobis(2-nitrobenzoic acid) - a reexamination. Analytical Biochemistry 94, 75-81.

Santos, E.S., Abreu, M.M., Nabais, C., Saraiva, J., 2009. Trace elements and activity of antioxidative enzymes in *Cistus ladanifer* L. growing on an abandoned mine area. Ecotoxicology 18, 860-868.

Santos, E.S., Abreu, M.M., Nabais, C., Magalhães, M.C.F., 2012. Trace element distribution in soils developed on *gossan* mine wastes and *Cistus ladanifer* L. tolerance and bioaccumulation. Journal of Geochemical Exploration 123, 45-51.

Santos, E.S., Abreu, M.M., Batista, M.J., Magalhães, M.C.F., Fernandes, E., 2014. Inter-population variation on the accumulation and translocation of potentially harmful chemical elements in *Cistus ladanifer* L. from Brancanes, Caveira, Chança, Lousal, Neves Corvo and São Domingos mines in the Portuguese Iberian Pyrite Belt. Journal of Soils and Sediments 14, 758-772.

Sharma, P., Dubey, R.S., 2005. Lead toxicity in plants. Brazilian Journal of Plant Physiology 17, 35-52.

Słomka, A., Libik-Koniecznyb, M., Kutaa, E., Miszalskib, Z., 2008. Metalliferous and non-metalliferous populations of *Viola tricolor* represent similar mode of antioxidative response. Journal of Plant Physiology 165, 1610-1619.

Stoeva, N., Bineva, T., 2003. Oxidative changes and photosynthesis in oat plants grown in as-contaminated soil. Bulgarian Journal of Plant Physiology 29(1-2), 87-95.

Sun, M., Zigman, S., 1977. An improved spectrophotometic assay for superoxide dismutase based on epinephrine autoxidation. Analytical Biochemistry 90,81-89.

Tavares, M.T., Sousa, A.J., Abreu, M.M., 2008. Ordinary kriging and indicator kriging inthe cartography of trace elements contamination in São Domingos mining site (Alentejo, Portugal). Journal of Geochemical Exploration 98, 43-56.

Teklić, T., Hancock, J.T., Engler, M., Paradicović, N., Cesar, V., Lepeduš, H., Štolfa, I., Bešlo, D., 2008. Antioxidative responses in radish (*Raphanus sativus* L.) plants stressed by copper and lead in nutrient solution and soil. Acta Biologica Cracoviensia 50(2), 79-86.

Verma, S., Dubey, R.S., 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Science 164, 645-655.

Wong, M.H., 2003. Ecological restoration of mine degraded soils with emphasis on metal contaminated soils. Chemosphere 50, 775-780.

Wong, D.W.S., Whitaker, J.R., 2003. Catalase, in: Whitaker, J.R., Voragen, A.G.J., Wong, D.W.S. (Eds), Handbook of food enzymology. Marcel Dekker, New York, pp. 389-401.

Yruela, I., 2005. Copper in plants. Brazilian Journal of Plant Phisiology 17. 145-156.

Zengin, F.K., Unzuroglu, O.M., 2005. Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. Acta Biologica Cracoviensia 47(2), 157-164.

5. COMPOSITION AND AROMATIC PROFILE OF EXTRACTS FROM CISTUS LADANIFER AND LAVANDULA PEDUNCULATA GROWING IN SÃO DOMINGOS MINING AREA

ABSTRACT

The aims of this study were to: i) characterize the phytochemical profile of the different extracts (water and hexane) from shoots of *Lavandula pedunculata* and *Cistus ladanifer* growing in soils from São Domingos mining area and a control area in the same climatic conditions; ii) quantify some of the major components of these extracts; and iii) evaluate the influence of potentially hazardous elements accumulated in the aerial part of the plants on the quality of plant-based products (aqueous and hexane extracts). Composite samples of soils, developed on mine wastes and/or host rocks, as well as shoots of *L. pedunculata* and *C. ladanifer* were collected in São Domingos mine (SE of Portugal) and in a reference area with non-contaminated soils (Corte do Pinto)

Total concentrations of potentially hazardous elements (Al, As, Cr, Cu, Mn, Sb and Zn) in soils, shoots and *L. pedunculata* infusions were determined. The extracts from *C. ladanifer* and *L. pedunculata* shoots were obtained by an accelerated solvent extractor, and the compounds were analysed by GC-MS. Extracts of both species were extracted with hexane (single extraction), while *L. pedunculata* was also subjected to a sequential extraction with water and hexane. Major components in all extracts were quantified.

Soils from São Domingos can be considered contaminated with As, Cu, Pb and Sb. In general, concentrations of the studied elements in shoots of both species (excepted Cr and Mn in *L. pedunculata* and Cr in *C. ladanifer*) collected in São Domingos mining area were higher than in plant shoots from non-contaminated area. Only concentrations of Zn in the infusions done with São Domingos plants were significantly different. However, in general, concentrations of potentially hazardous elements in infusions were small. The major component in the *C. ladanifer* extracts was viridiflorol, while in *L. pedunculata* extracts was camphor, independently of the sample. Extraction method (single and sequential) and solvent did not affect the major components identified in the *L. pedunculata* extracts. However, for other compounds an influence of the solvent used in their extraction was observed. In both species, slight variability intra and interpopulation was observed in the qualitative composition and concentrations of major components, but no relationship was found between these components and the concentrations of the studied potentially hazardous elements in shoots. Extracts obtained from *C. ladanifer* and *L. pedunculata* growing in São Domingos mining area had valuable compounds.

High concentrations of potentially hazardous elements in soils did not affect the quality of the plant-based products. Phytostabilisation of mining areas with these species can provide economic return by the exploration of these products.

KEYWORDS Camphor • Lavander infusion • Potentially hazardous elements • São Domingos mine • Valuable extracts • Viridiflorol

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INTRODUCTION

In the Portuguese Iberian Pyrite Belt, several mining areas are abandoned and show significant environmental impacts related to great volumes of different tailings; high total concentrations of potentially hazardous elements in mine wastes, soils, sediments and waters (Álvarez-Valero et al., 2008; Matos and Martins, 2006; Pérez-López et al., 2008). Generally, these mine wastes and the adjacent soils, as well as soils developed on mine wastes have unfavourable characteristics to plant development, namely low fertility and high total concentrations of potentially hazardous elements, small water holding capacity and weak structure (Abreu and Magalhães, 2009; Wong, 2003). However, some autochthones plant species with refereed aromatic and medicinal properties (*e.g. Rosmarinus officinalis* and some species of the genus *Cistus*, *Lavandula* and *Erica*) are able to naturally colonize these substrata improving their physical, chemical and biological characteristics and, consequently, contributing to the natural rehabilitation of the mining area (Abreu and Magalhães, 2009; Abreu et al., 2008, 2012a,b; Pérez-López et al., 2014; Santos et al., 2014). These species also grow in non-contaminated areas, being the populations from the mining areas considered specific tolerant ecotypes, which developed effective tolerance mechanisms (Abreu et al. 2012a; Santos et al., 2009, 2012).

Continuous degradation of land resources by mining activities can reduce the agricultural areas, while the rehabilitation of mining areas is essential and a priority because those are sources of contamination and environmental and health risk. The rehabilitation of abandoned mines by phytostabilisation involves several ecological improvements (Abreu and Magalhães, 2009; Mendez and Maier, 2008) but, nowadays, the economical approaches are also essential. Thus, the rehabilitation of non-productive and contaminated soils/mine wastes can be a niche for agriculture/forestry exploration, by implementation of phytostabilisation, in order to produce plant-based products. In fact, the growth of aromatic and medicinal crops (*e.g. Anethum graveolens* L., *Mentha x piperita* L., *Ocimum basilicum* L. and *Lavandula angustifolia* Mill.) on agricultural soils enriched with Cd, Cu and Pb is considered an economic and environmental feasible option (Zheljazkov and Nielsen, 1996; Zheljazkov et al., 2006, 2008). According to the same authors, the essential oil yield can be reduced with the plant growth on contaminated soils and consequently increase of potentially hazardous elements in shoots but, in the process of the oil extraction, these elements remain in the extracted plant residues limiting their quantities in the commercial oil product.

Several plants extracts are used for perfumery, pharmaceutical and cosmetics as well as food addictive (Raut and Karuppayil, 2014). *Cistus ladanifer* L. is reported by its medicinal (*e.g.* antioxidant, antifungal, antibacterial, antidiarrheic and anti-inflammatory) (Andrade et al., 2009; Attaguile et al., 2000; Barros et al., 2013; Greche et al., 2009; Zidane et al., 2013) and odoriferous importance for fragrance industry (Gomes et al., 2005; Teixeira et al., 2007). The essential oil of this species also showed herbicidal activity in vitro bioassays presenting great potential as natural herbicide for crop protection (Verdeguer et al., 2011). Nevertheless, *C. ladanifer* is a very poorly exploited species in Portugal although in France and Spain markets have great importance. The use of *Lavandula* species as aromatic, for food and fragrance industries, and medicinal (*e.g.* anti-inflammatory, antibacterial,

digestive and calming) plant, especially in the form of infusions, is reported (Cavanagh and Wilkinson, 2002; Figueiredo et al., 2014). Oils of these species are also indicated as a potential natural biopesticide (González-Coloma et al., 2011). In particular, *Lavandula pedunculata* (Mill.) Cav. Present antifungal and antioxidant activity being, additionally, a potential source of active metabolites with a positive effect on human health (Costa et al., 2013; Matos et al., 2009; Zuzarte et al., 2009).

Several studies have been developed on some mining areas from the Iberian Pyrite Belt aiming to evaluate the elements accumulation in tolerant plants and their implications for phytostabilisation (Abreu et al., 2008; Anawar et al., 2011; de la Fuente et al., 2010; Freitas et al., 2004; Santos et al., 2012). Nevertheless, little information is available concerning the quality of plants as source of important phytochemicals and/or the public safety risk of these products derived from plants growing in contaminated areas (Zheljazkov et al., 2006, 2008).

In order to characterize and valorise some autochthones species, which are used in rehabilitation processes of mining areas, as new sources of bioactive substances, the aims of this study were to: i) characterize the phytochemical profile of the different extracts (aqueous and hexane extraction) from shoots of *Lavandula pedunculata* and *Cistus ladanifer* growing in soils from São Domingos mining area and a control area in the same climatic conditions; ii) quantify some of the major components of these extracts; and iii) evaluate the influence of potentially hazardous elements accumulated in the aerial part of the plants on the quality of the plant-based products (infusions and extracts).

MATERIALS AND METHODS

Site characterization

Two areas, under Mediterranean conditions (Alentejo region, SE of Portugal), were selected to this study: a mining area with multielementar contamination − São Domingos (SD), and a reference area with non-contaminated soils − Corte do Pinto (CP). In both areas, *Lavandula pedunculata* (Mill.) Cav. (sin. *Lavandula pedunculata* subsp. *Sampaioana* (Rozeira) Franco, *Lavandula stoechas* subsp. *lusitanica* (Chaytor) Rozeira, *Lavandula stoechas* subsp. *pedunculata* (Mill.) Rozeira and *Lavandula sampaioana* (Rozeira) Rivas Mart., T.E. Díaz & Fern. Gonz.) and *Cistus ladanifer* L. are representative species of the vegetation community in both areas. According to Thornthwaite classification, the climate in these areas is semiarid, mesothermic.

São Domingos is an old copper mine located in the Iberian Pyrite Belt, which is abandoned since 1960. The mining activities occurred since pre-roman period and in the modern times from the middle of the XIX century till the 60's of the XX century (Quental et al., 2002). As a result of the different periods and techniques of exploitation, large amounts of wastes from ore processing or extracting were produced and spread, irregularly, along the mine area (Álvarez-Valero et al., 2008; Pérez-López et al., 2008, Quental et al., 2003). On some of these materials were developed Incipient soils (Spolic Technosol Toxic − IUSS Working Group WRB, 2007), which present high total concentrations of several potentially hazardous elements (Abreu et al., 2008, 2012a,b; Freitas et al., 2004; Pérez-López et al., 2014; Santos et al., 2012, 2014, Tavares et al., 2008). In the mining area, there are also some soils developed on schists and greywackes (Leptosols Lithic; IUSS Working Group WRB, 2007),

which are influenced by adjacent tailings and/or acid mine drainage. Corte do Pinto area is located about 4 km to the north of São Domingos mine whose soils are also classified as Leptosols Lithic (IUSS Working Group WRB, 2007) but without any contamination.

Soils and plants analysis

Several sampling areas were selected in the two study areas in order to include representative soils where *L. pedunculata* (three in SD mining area and three in CP) or *C. ladanifer* (three in SD mining area and three in CP) were growing. In each sampling area (≈10 m^2 each), composite samples of shoots of *L. pedunculata* and *C. ladanifer* were collected during the flowering period (April 2012) as well as soils surrounding the rhizosphere system of all harvested plants (≈3 kg of homogenate; < 20 cm of depth). Soil samples were air-dried, homogenised, and sieved. Classical characterization of soils (fraction < 2 mm) was carried out (Póvoas and Barral, 1992): pH and electrical conductivity in water suspension (1:2.5 *m*/*V*); organic carbon (Tinsley method); extractable P and K (Egner-Riehm method) and total nitrogen (Kjeldahl method). The total concentrations of Al, As, Cr, Cu, Mn, Pb, Sb and Zn in soils (fraction < 2 mm) were determined by ICP and INAA, after acid digestion (perchloric acid + nitric acid + hydrochloric acid + hydrofluoric acid; Actlabs ISO/IEC 17025; Activation Laboratories, 2015a).

Plant samples were washed, dried (40 °C), homogenised and stored in the dark at room temperature until analysis. For multielementar determination, shoots were finely ground and analysed by ICP-MS after ashing (475 °C) and nitric acid digestion (Activation Laboratories, 2015b).

For obtaining the extracts, the dried plant material (SD1−SD5 and CP1) was ground in a wood chipper/leaf shredder. The extracts were obtained with an accelerated solvent extractor (ASE, Dionex). For this, 3 g of *L. pedunculata* shoots or 4 g of *C. ladanifer* shoots were placed in 11 mL-stainless steel cells. Plant samples were treated with hexane (single extraction of both plant species) or with water and then with hexane (sequential extraction of *L. pedunculata*), at 100 ºC, 2000 psi, for 30 min, and one extraction cycle. Compounds from water extracts were ultrasonically extracted with hexane (1:1 *V*/*V*) for one hour. Extracts were stored in a glass flask at −18 ºC until their analysis. The solvents (water and hexane) were selected due to the sustainability of the process and extraction efficiency. Sequential extraction was done in order to evaluate the recover efficiency of the valuable compounds with both extractant solutions.

Extracts were analysed by gas chromatography (Model 450 GC, Agilent Technologies) coupled to mass spectrometry (Model 220 MS, Agilent Technologies). Before analysis, a mix of deuterated internal standards, containing 1,4-dichlorobenzene-d4, acenaphthene-d10, chrysene-d12, naphthalene-d8, perylene-d12 and phenanthrene-d10 (Internal Standards Mix 33, Dr. Ehrenstorfer), were added to the extracts at 0.5 mg/L as a constant concentration. Chromatographic separations were performed by a FactorFour VF-5ms EZ-Guard capillary column (30 m x 0.25 mm x 0.25 µm; Agilent Technologies) operated with the following oven temperature program: 40 ºC (held for 10 min) to 180 ºC (held for 10 min), at 3 ºC/min. Helium was used as carrier gas, at a constant flow of 1 mL/min. The injector was operated at 250 ºC in split/splitless mode. The mass spectrometer operated

in full scan mode. Ionization of the molecules was carried out by electron impact and the ion trap temperature was fixed at 220 ºC.

The composition of the extracts was expressed in percentage values calculated by the normalization method from the GC peak areas, without using correction factors, and as mean values of two injections from each extract. The component identification was made by comparison of mass spectra using NIST98 spectral library, their retention indices, pure reference compounds and literature data. The linear retention indices were determined relative to the retention times obtained for a standard mixture containing a series of n-alkanes C9−C18.

For the quantitative analysis of the major components in the extracts, a calibration curve was obtained by injection of a standard containing a mixture of fenchone, verbenone, camphene, camphor and α-pinene. The calibration standards were prepared in hexane, at several concentrations: 0.1, 1, 5, 10, 25, 50, 75, 100 and 150 mg/L. Internal standards were also added in the same concentration as for the plant samples (0.5 mg/L). The results were expressed in mg of each compound per kg of dried shoots.

Plant infusions were prepared with 5 g of *L. pedunculata* shoots (SD1−SD3 and CP1−CP3; particle size < 4 mm) and 100 ml of boiling water during 5 min of contact. Same elements than in soils were determined by Flame-AAS (Al, Cr, Cu, Mn, Pb and Zn) and GF-AAS (As and Sb).

Data analysis

Data were analysed with the statistical programme SPSS v18.0 for Windows namely: concentration of the major compounds in the plant extracts by one way ANOVA and the Duncan test ($p < 0.05$) and concentrations of the potentially hazardous elements in *L*. pedunculata infusions by non-parametrically using Kruskal–Wallis ANOVA by Ranks test. Bivariate Pearson correlations were used to correlate concentrations of the elements in the plant shoots and the major compounds quantified ($r > 0.95$). Quality control of the analyses was made by analytical replicate samples, use of certified standards and blanks.

RESULTS AND DISCUSSION

Characteristics of the soils and plants

Soils from São Domingos were developed on several mine wastes without or with different proportions of host rocks. This fact contributed to a great heterogeneity in their chemical characteristics (Table 1 and Fig. 1). The pH and concentrations of organic C and NPK the soils from the mine area were lower than those of Corte do Pinto (Table 1). Although some soils from São Domingos are developed on schist and sediments, their intense influence by acid mine drainage contribute to acid pH values. Moreover, the mixture of considerable proportion of host rocks with *gossan* led to the neutral pH of soil sample SD1. Low electrical conductivities and poor fertility were obtained in soils from both areas (Table 1).

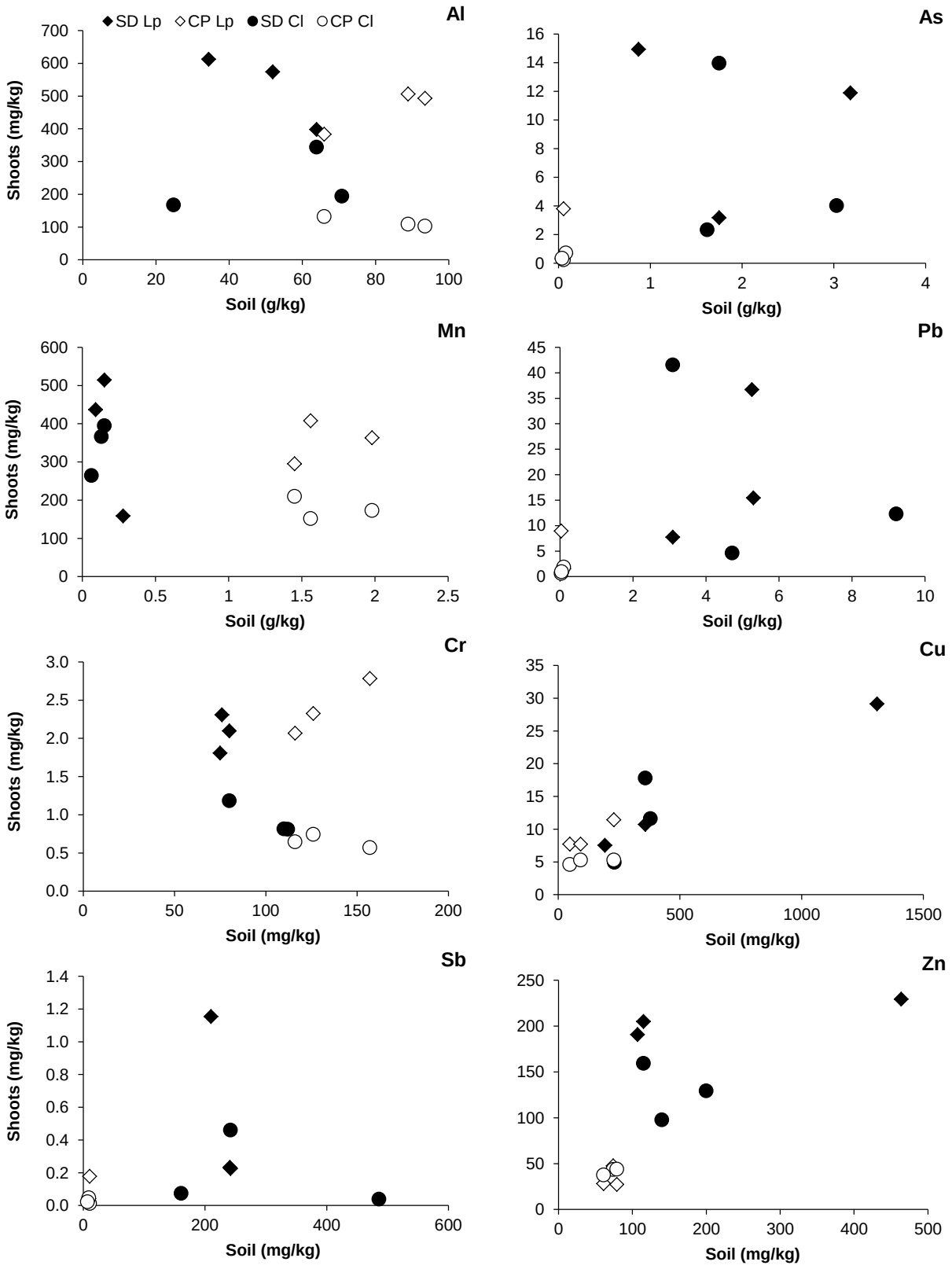

Fig. 1 Concentrations of potentially hazardous elements in soil (total fraction) and shoots of *Lavandula pedunculata* (Lp) and *Cistus ladanifer* (Cl) collected in the São Domingos (SD) and Corte do Pinto (CP).

Table 1 Chemical characteristics of the soils from São Domingos mine (SD) and Corte do Pinto (CP) areas and respective plant species collected.

	SD1	**SD2**	**SD3**	**SD4**	**SD5**	**CP1**	**CP2**	**CP3**
Plant species	*L. pedunculata*	*L. pedunculata*	*L. pedunculata* *C. ladanifer*	*C. ladanifer*	*C. ladanifer*	*L. pedunculata* *C. ladanifer*	*L. pedunculata* *C. ladanifer*	*L. pedunculata* *C. ladanifer*
pH (H_2O)	6.7	4.4	3.5	4.3	4.3	6.6	7.1	7.1
EC (µS/cm)	383	—	274	—	603	104	246	423
N_{Total} (g/kg)	0.7	1.0×10^{-2}	1.5	39.9	1.1	1.3	1.5	1.8
C_{Org} (g/kg)	9.4	125.2	21.0	12.0	14.5	14.3	16.3	22.5
P_{Ext} (mg/kg)	8.7	0.2	<0.9	1.3	<0.9	6.4	13.9	17.1
K_{Ext} (mg/kg)	100.5	65.6	56.5	83.3	143.2	98.0	165.2	128.7

EC: electrical conductivity in water 1:2.5 (*m*:*V*); C_{org}: Organic C; P_{Ext}: Extractable P; K_{Ext}: Extractable K

The total concentrations of As, Cu, Pb, Sb and Zn were higher in soils from São Domingos than from Corte do Pinto (Fig. 1), while the opposite was obtained for Al, Cr and Mn. Despite of the variability, the total concentrations of the potentially hazardous elements in the soils from São Domingos are in the same range than those reported for mine wastes and/or soils developed on mine wastes from the same mine area (Abreu et al., 2012a,b; Álvarez-Valero et al., 2008; Anawar et al., 2011, 2013; Durães et al., 2015; Freitas et al., 2004, 2009; Pérez-López et al., 2008; Santos et al., 2012, 2014; Tavares et al., 2008).

Soils from São Domingos can be considered contaminated with As, Cu, Pb and Sb for agriculture and residential/parkland uses (maximum allowed values (MAV): 12 mg As/kg, 63 mg Cu/kg, 70−140 mg Pb/kg and 20 mg Sb/kg, depending on land use; CCME, 2007) while only one sample exceeded the MAV for Zn (200 mg/kg; CCME, 2007).Total concentrations of As in soils from Corte do Pinto were above the MAV, however those are not considered as contaminated. In fact, these amounts are associated with the natural composition of the parent materials (schists and greywackes) from which soils were developed (Abreu et al., 2008; Tavares et al., 2008).

Interpopulation variation was observed for the concentrations of the potentially hazardous elements in the shoots of *C. ladanifer* and *L. pedunculata* (Fig. 1), as well as in the populations of both species from São Domingos (intrapopulation variability). This heterogeneity was already reported to *C. ladanifer* (Santos et al., 2012, 2014 and references therein). Concentrations of the studied elements (excepted Cr and Mn in *L. pedunculata* and Cr in *C. ladanifer*) in the shoots of both species collected in São Domingos mine were higher than in those from non-contaminated area. In general, the same plant parts of the two species collected in São Domingos or other mining areas under arid and semiarid conditions presented a wide range of concentrations for some of the studied elements (Santos et al., 2014 and references therein; de la Fuente et al., 2010) being, in some cases, similar compared to those obtained in the present study. Nevertheless, these differences can be explained by seasonal and temporal variations in plants and the analysed plant part.

Infusions and extracts of Lavandula pedunculata: Component identification and quantification

Independently of the contamination soil level and the concentrations of the potentially hazardous elements in shoots, the concentrations of Al, As, Cr, Pb and Sb in the infusions were below the detection limit of the equipment measurement (mg/L − < 0.5, 5.0 x 10^{-4}, 0.05, 0.25 and 0.03, respectively). No significant differences were obtained between the concentrations of Cu (mg/L − SD: 0.06−0.13, CP: 0.07−0.10 depending on sample) and Mn (mg/L –SD: 0.52−4.54, CP: 2.17−3.14 depending on sample) in infusions done with plants from the two studied areas. Nonetheless, Zn concentrations were higher in the infusions done with São Domingos plants (0.57−1.45 mg/L) than in those made with plants collected in the non-contaminated area (0.21−0.25 mg/L).The concentrations of the studied elements were in the same range than those reported by Görür et al. (2011) for black and green teas, as well as for several herbal infusions (Altıntıg et al., 2014) consumed in Turkey. In this way, the consumption of infusions done with *L. pedunculata* from studied areas did not have health risk.

In the *L. pedunculata* extracts, obtained in the single extraction with hexane, were identified 34 compounds accounting between 79 and 89 % of the total identified compounds (Table 2). Camphor was the major component in all extracts (Table 2). Fenchone, eucalyptol, verbenone, bornyl acetate, borneol and linalool oxide cis also showed considerable amounts in all extracts. These compounds present economic interest. In fact, camphor has been used as an antibacterial and antifungal substance and improving sleep quality being also a natural flavouring agent and an ingredient for food processing (Chu and Kemper, 2001). According to the same authors, fenchone also shows a significant antifungal activity and Cavanagh and Wilkinson (2002) indicated that camphor plants are used as insect repellents. Although considerable amounts of eucaliptol had been identified in *Lavandula* samples, at economic level cannot be significant its extraction because the essential oil obtained from *Eucaliptus globulus* Labill. biomass residues (S of Portugal) had around 46 % of eucalyptol (Gonçalves et al., 2013), being more profitable. Elaissi et al. (2012) also reported significant amounts of eucalyptol (between 4.5 and 70 %) in oils extracted from different eucalyptus species from Tunisia. Verbenone, bornyl acetate, borneol and linalool-family are compounds suggested for perfumery and household fragrances. Verbenone, bornyl acetate and borneol also present medicinal (*e.g.* antimicrobial and antispasmodic) and insect repellent properties (Phytochemical and ethnobotanical databases, 2015).

Although with amounts lesser than 1 % (Table 2), other valuable compounds were identified with great interest for medicine (*e.g.* eugenol: antibacterial, spasmolytic and anesthetic effect; coumarim and caryophyllene oxyde: anti-inflammatory; α- and β-pinene: antifungal) as well as for fragrance of cosmetics or household cleaners (*e.g.* linalyl acetate). Therefore, they should be highlighted.

Some variation was obtained in the qualitative composition of the *L. pedunculata* extracts obtained by single extraction but, in general, it was not clear the differentiation between populations (Table 2) and, consequently, soil contamination level and concentrations of the potentially hazardous elements in shoots (Fig. 1). Concerning the concentrations of the some major components (Table 3), some significant differences were obtained among plant samples (except for verbenone) but no correlation was obtained with the potentially hazardous elements in shoots. Thus, sample SD2 had the highest concentration of fenchone while sample from non-contaminated area (CP1) presented the highest concentration of camphor. Samples from SD3 and CP1 presented much more camphene. The use of composite samples of shoots affects the composition and, especially, the concentration of the compounds in the extracts (Masetto et al., 2015; Raut and Karuppayil, 2014) justifying these variations.

In the aqueous extract (first step of the sequential extraction) were identified 27 compounds, independently of the plant samples, while in the following step (with hexane) were identified 26 compounds (Table 4). The total fraction of the identified compounds varied between 52 and 85 % depending on the sample and extraction step/solvent.

As for single extraction, major component in both extracts was camphor (Table 4). Fenchone and verbenone also had considerable amounts, especially in the aqueous extract, as well as borneol. It should be noted that, in general, α- and β-pinene, bornyl acetate viridiflorol, hinesol and longiverbenone also had considerable amounts but only in the hexane extract. As was stated by

Table 2 Chemical composition (%) of the extract, obtained in a single extraction with hexane, from *Lavandula pedunculata* shoots collected in São Domingos mine (SD) and Corte do Pinto (CP; non-contaminated area) areas (mean ± σ; *n* = 3).

LRI	Compound identified	SD1	SD2	SD3	CP1
902	α-Thujene	0.43 ± 0.05	0.24 ± 0.02	0.80 ± 0.10	0.56 ± 0.04
916	α-Pinene	0.42 ± 0.04	1.01 ± 0.16	0.45 ± 0.08	0.27 ± 0.05
935	Camphene	2.33 ± 0.09	1.49 ± 0.20	4.43 ± 0.64	1.75 ± 1.12
940	β-Pinene	0.25 ± 0.08	0.35 ± 0.06	0.31 ± 0.08	0.26 ± 0.07
1020	1,3,8-p-Menthatriene	0.25 ± 0.01	0.23 ± 0.01	0.30 ± 0.02	0.25 ± 0.01
1027	Eucalyptol	9.63 ± 2.18	6.02 ± 0.21	1.48 ± 0.14	6.91 ± 0.39
1068	Linalool oxide cis	1.52 ± 0.24	1.45 ± 0.08	1.25 ± 0.06	0.96 ± 0.05
1076	Camphenilone	0.51 ± 0.10	0.28 ± 0.01	0.96 ± 0.08	0.81 ± 0.03
1082	Fenchone	13.68 ± 0.86	29.49 ± 0.98	6.17 ± 0.41	16.48 ± 0.60
1098	Linalool	—	0.23 ± 0.02	—	—
1113	exo-Fenchol	0.51 ± ± 0.07	1.18 ± 0.07	0.42 ± 0.04	0.55 ± 0.04
1121	6-Camphenol	0.09 0.02	0.21 ± 0.04	0.14 ± 0.02	0.08 ± 0.02
1131	β-Pinone (Nopinone)	0.06 ± 0.01	0.05 ± 0.004	0.06 ± 0.004	0.05 ± 0.01
1134	Isopinocarveol	0.12 ± 0.02	0.26 ± 0.02	0.17 ± 0.03	0.11 ± 0.02
1139	Camphor	39.45 ± 2.03	23.15 ± 1.24	44.96 ± 8.60	45.16 ± 2.41
1156	Pinocarvone	0.08 ± 0.02	0.25 ± 0.03	0.09 ± 0.02	0.10 ±0.004
1166	Borneol	1.92 ± 0.30	1.44 ± 0.09	2.45 ± 0.59	2.34 ± 0.11
1182	p-Cymen-8-ol	0.79 ± 0.14	1.00 ± 0.06	0.95 ± 0.04	0.81 ± 0.04
1190	Myrtenol	0.06 ± 0.02	0.17 ± 0.06	0.05 ± 0.01	0.08 ± 0.03
1200	Verbenone	4.14 ± 0.69	4.58 ± 0.26	5.20 ± 0.23	3.76 ± 0.25
1221	exo-2-Hydroxycineole	0.12 ± 0.01	0.10 ± 0.01	0.05 ± 0.004	0.09 0.01±
1248	Lynalylacetate	0.26 ± 0.05	0.47 ± 0.03	0.32 ± 0.01	0.23 ± 0.01
1259	dl-Camphoroquinone	0.36 ± 0.13	0.42 ± 0.03	0.40 ± 0.04	0.41 ± 0.02
1266	(1R,2R,3S,5R)-(-)-Pinanediol	0.46 ± 0.10	1.04 ± 0.10	0.55 ± 0.02	0.60 ±0.04
1280	Bornyl acetate	2.23 ± 0.19	1.11 ± 0.08	5.05 ± 0.32	4.12 ±0.20
1343	Eugenol	0.12 ± 0.02	0.23 ± 0.02	0.17 ± 0.07	0.10 ±0.003
1367	Exo-ketoborneol	0.51 ± 0.11	0.45 ± 0.04	0.60 ± 0.05	0.39 ± 0.01
1424	Coumarin	0.09 ± 0.02	0.12 ± 0.01	0.16 ± 0.02	0.04 ±0.004
1482	Aromadendrene	0.20 ± 0.09	0.29 ± 0.03	0.11 ± 0.06	0.15 ±0.04
1583	Viridiflorol	0.34 ± 0.05	0.13 ± 0.02	0.28 ± 0.02	0.16 ±0.004
1596	Globulol	0.05 ± 0.02	0.03 ± 0.01	0.06 ± 0.02	0.01 ±0.01
1601	Caryophyllene oxide	0.14 ± 0.03	0.04 ± 0.01	0.06 ± 0.01	0.07 ±0.01
1610	Hinesol	0.29 ± 0.04	0.55 ± 0.15	0.28 ± 0.01	0.29 ±0.01
1635	tau-Cadinol	0.06 ± 0.02	0.13 ± 0.03	0.05 ± 0.02	0.12 ±0.04
1666	Longiverbenone	0.24 ± 0.06	—	0.09 ± 0.02	0.05 ±0.02
Total identified compounds		83.02 ± 8.17	79.41 ± 4.40	80.06 ± 12.08	89.24 ± 5.83

LRI: Linear retention indices determined with homologous series of n-alkanes

Table 3 Concentration of some major components (mg/kg) in extracts, obtained with different extraction solutions, from *Lavandula pedunculata* shoots collected in São Domingos mine (SD) and Corte do Pinto (CP; non-contaminated area) (mean ± σ; *n* = 3).

	mg/kg			
Samples	**Camphene**	**Camphor**	**Fenchone**	**Verbenone**
		Single extraction with hexane		
SD1	106.1 ± 12.8^{b}	1501.5 ± 365.1^{bc}	503.0 ± 112.3^{c}	185.2 ± 59.6^{a}
SD2	79.4 ± 11.6^{b}	1110.6 ± 232.6^{c}	1446.9 ± 312.3^{a}	258.9 ± 51.3^{a}
SD3	182.9 ± 33.5^{a}	2039.9 ± 238.6^{b}	232.8 ± 30.4^{c}	246.3 ± 22.2^{a}
CP1	155.8 ± 35.0^{a}	2646.7 ± 412.5^{a}	921.2 ± 114.9^{b}	245.3 ± 42.5^{a}
		1st step of sequential extraction with water		
SD1	2.8 ± 1.0^{b}	1784.3 ± 562.5^{a}	520.1 ± 164.2^{b}	389.3 ± 118.6^{a}
SD2	1.9 ± 0.8^{b}	741.9 ± 163.2^{b}	911.67 ± 208.5^{a}	310.3 ± 83.4^{a}
SD3	3.7 ± 0.8^{ab}	1272.3 ± 298.6^{ab}	143.2 ± 30.04^{c}	245.0 ± 58.4^{ab}
CP1	5.1 ± 2.2^{a}	1064.9 ± 180.7^{b}	304.3 ± 34.3^{b}	155.1 ± 23.0^{b}
		2nd step of sequential extraction with hexane		
SD1	4.2 ± 0.4^{a}	179.0 ± 32.2^{a}	21.6 ± 4.1^{b}	39.2 ± 7.0^{a}
SD2	3.8 ± 0.2^{ab}	113.6 ± 5.8^{b}	5.2 ± 1.5^{b}	31.1 ± 0.6^{a}
SD3	2.9 ± 0.9^{b}	99.9 ± 42.7^{b}	64.8 ± 29.6^{a}	54.0 ± 30.9^{a}
CP1	4.9 ± 0.4^{a}	149.6 ± 5.3^{ab}	21.3 ± 1.3^{b}	26.2 ± 1.0^{a}

Values for each compound and extraction/step of extraction followed by a different letter are significantly different ($p < 0.05$)

Table 4 Chemical composition (%) of the extracts, obtained by sequential extraction with water and hexane, from *Lavandula pedunculata* shoots collected in São Domingos mine (SD) and Corte do Pinto (CP; non-contaminated area) (mean ± σ; *n* = 3).

LRI	Compound identified	SD1	SD2	SD3	CP1	SD1	SD2	SD3	CP1
		1st step of sequential extraction with water				*2nd step of sequential extraction with hexane*			
916	α-Pinene	—	—	—	—	3.03 ± 0.70	2.81 ± 1.40	4.10 ± 0.50	3.12 ± 1.55
935	Camphene	0.06 ± 0.02	0.05 ± 0.01	0.11 ± 0.01	0.12 ± 0.05	0.73 ± 0.11	0.60 ± 0.16	1.00 ± 0.17	0.80 ± 0.34
940	β-Pinene	—	—	—	—	1.42 ± 0.62	0.89 ± 0.13	1.52 ± 0.14	1.26 ± 0.57
1020	1,3,8-p-Menthatriene	0.01 ± 0.01	0.03 ± 0.005	0.02 ± 0.004	0.02 ± 0.003	0.11 ± 0.01	0.14 ± 0.02	0.07 ± 0.01	0.12 ± 0.05
1027	Eucalyptol	7.06 ± 0.50	4.04 ± 0.05	0.94 ± 0.18	2.91 ± 0.49	1.53 ± 0.19	0.90 ± 0.21	—	0.67 ± 0.30
1068	Linalool oxide cis	2.52 ± 1.15	2.89 ± 0.33	2.15 ± 0.23	1.01 ± 0.18	0.47 ± 0.08	0.78 ± 0.03	0.45 ± 0.09	0.28 ± 0.10
1076	Camphenilone	0.84 ± 0.09	0.44 ± 0.03	1.35 ± 0.16	0.76 ± 0.16	0.13 ± 0.03	0.05 ± 0.02	0.17 ± 0.07	0.18 ± 0.08
1082	Fenchone	11.83 ± 1.93	27.63 ± 1.91	4.09 ± 0.07	9.18 ± 2.02	3.05 ± 0.38	10.02 ± 1.20	1.16 ± 0.15	3.40 ± 1.58
1098	Linalool	—	0.12 ± 0.01	—	—	—	0.21 ± 0.19	—	—
1113	exo-Fenchol	0.72 ± 0.05	1.09 ± 0.12	0.62 ± 0.04	0.42 ± 0.08	0.20 ± 0.17	1.05 ± 0.11	—	—
1121	6-Camphenol	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.04 ± 0.01	0.14 ± 0.06	—	—
1131	β-Pinone (Nopinone)	0.14 ± 0.02	0.10 ± 0.01	0.10 ± 0.01	0.06 ± 0.01	—	—	—	—
1134	Isopinocarveol	0.12 ± 0.02	0.22 ± 0.03	0.17 ± 0.02	0.07 ± 0.03	0.13 ± 0.02	0.39 ± 0.07	0.26 ± 0.05	0.05 ± 0.03
1139	Camphor	42.76 ± 1.09	23.66 ± 1.53	55.12 ± 3.84	35.52 ± 6.75	24.55 ± 3.57	14.55 ± 1.91	25.42 ± 4.59	22.54 ± 9.97
1156	Pinocarvone	0.04 ± 0.01	0.09 ± 0.004	0.02 ± 0.01	0.02 ± 0.004	—	—	—	—
1166	Borneol	2.82 ± 0.24	1.47 ± 0.14	3.36 ± 0.33	1.81 ± 0.34	3.49 ± 0.43	3.59 ± 1.03	5.17 ± 1.30	1.24 ± 0.59
1179	Eucarvone	0.34 ± 0.04	0.15 ± 0.01	0.01 ± 0.001	0.06 ± 0.01	—	—	—	—
1182	p-Cymen-8-ol	1.42 ± 0.12	1.64 ± 0.18	1.51 ± 0.06	0.81 ± 0.15	1.29 ± 0.10	2.24 ± 0.28	1.94 ± 0.40	1.04 ± 0.45
1190	Myrtenol	0.15 ± 0.02	0.30 ± 0.05	0.16 ± 0.01	0.11 ± 0.09	0.23 ± 0.02	0.62 ± 0.29	0.33 ± 0.10	0.11 ± 0.05
1200	Verbenone	8.22 ± 0.60	8.75 ± 1.00	9.14 ± 0.79	4.33 ± 0.71	4.11 ± 0.51	6.44 ± 0.30	5.37 ± 1.01	3.04 ± 1.15

Continued on next page

Table 4 *Continued*

LRI	Compound identified	SD1	SD2	SD3	CP1	SD1	SD2	SD3	CP1
		1st step of sequential extraction with water				*2nd step of sequential extraction with hexane*			
1221	exo-2-Hydroxycineole	0.34 ± 0.01	0.27 ± 0.03	0.12 ± 0.03	0.12 ± 0.02	0.30 ± 0.03	0.92 ± 0.13	0.57 ± 0.11	0.13 ± 0.09
1248	Lynalylacetate	0.21 ± 0.01	0.18 ± 0.02	0.14 ± 0.01	0.09 ± 0.02	0.15 ± 0.02	0.29 ± 0.01	0.27 ± 0.04	0.27 ± 0.23
1259	dl-Camphoroquinone	0.60 ± 0.05	0.70 ± 0.06	0.61 ± 0.07	0.48 ± 0.11	0.29 ± 0.07	0.83 ± 0.16	0.30 ± 0.02	0.24 ± 0.08
1266	(1R,2R,3S,5R)-Pinanediol	0.69 ± 0.05	1.34 ± 0.14	0.64 ± 0.07	0.52 ± 0.10	0.37 ± 0.02	1.15 ± 0.21	0.52 ± 0.06	0.37 ± 0.16
1280	Bornyl acetate	0.18 ± 0.03	0.10 ± 0.03	0.62 ± 0.05	0.42 ± 0.08	2.12 ± 0.31	1.45 ± 0.41	5.05 ± 1.05	3.39 ± 1.67
1343	Eugenol	0.17 ± 0.06	0.42 ± 0.04	0.18 ± 0.01	0.09 ± 0.04	0.28 ± 0.04	0.70 ± 0.06	0.39 ± 0.09	0.05 ± 0.06
1367	Exo-ketoborneol	0.66 ± 0.19	0.48 ± 0.06	0.46 ± 0.06	0.22 ± 0.08	0.87 ± 0.10	0.81 ± 0.15	1.16 ± 0.30	0.12 ± 0.08
1424	Coumarin	0.25 ± 0.01	0.35 ± 0.04	0.35 ± 0.04	0.09 ± 0.03	—	—	—	—
1482	Aromadendrene	—	—	—	—	0.24 ± 0.04	0.44 ± 0.10	0.13 ± 0.06	0.16 ± 0.04
1665	Viridiflorol	0.06 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	—	2.48 ± 0.36	0.72 ± 0.22	1.87 ± 0.51	0.81 ± 0.52
1601	Caryophyllene oxide	0.09 ± 0.02	0.19 ± 0.03	0.13 ± 0.04	—	0.68 ± 0.44	0.86 ± 0.50	0.29 ± 0.09	0.19 ± 0.05
1610	Hinesol	—	—	—	—	1.84 ± 0.45	3.16 ± 1.00	1.38 ± 0.35	1.24 ± 0.61
1628	tau-Cadinol	—	—	—	—	0.56 ± 0.06	0.93 ± 0.35	0.51 ± 0.07	1.00 ± 0.15
1635	Longiverbenone	—	—	—	—	2.36 ± 0.30	0.76 ± 0.27	0.44 ± 0.16	0.22 ± 0.08
Total identified compounds		84.31 ± 6.8	78.94 ± 6.1	84.53 ± 6.6	60.86 ± 12.2	62.06 ± 9.19	65.72 ± 10.96	68.15 ± 11.50	51.82 ± 20.64

LRI: Linear retention indices determined with homologous series of n-alkanes

Zuzarte et al. (2009) to the essential oils of *L. pedunculata*, the aqueous extract from all the studied samples (SD and CP) can have a significant antifungal activity due to a synergistic effect among the different compounds with this proprietary (*e.g.* fenchone and camphor). Despite of the homogeneity of the compounds in the main fraction in the *Lavandula* extracts, some components depended on the sample. Other compounds were also detected but only in some samples (*e.g.* Aqueous extract − caryophyllene oxide and viridiflorol in plant samples from the mine area; Hexane extract − exo-Fenchol and 6-Camphenol in samples SD1 and SD2).

Due to the difficult systematic position of *L. pedunculata*, the comparison of the data can be quite complex but most of the components identified in this study were also reported by several authors for the same species (Costa et al., 2013; Figueiredo et al., 2014; Matos et al., 2015; Zuzarte et al., 2009) and for others *Lavandula* species (*Lavandula angustifolia* Mill*.,* Danh et al., 2012; *Lavadula luisieri* (Rozeira) Rivas Mart., González-Coloma et al., 2011) independently of the extraction method. In general, oils derived from *L. stoechas* (which can be included as subsp. synonymous of *L. pedunculata*) have high camphor levels what is in agreement with the data obtained in this study. For instance, the composition of the oils obtained from *L. stoechas* subsp. *lusitanica* and *L. pedunculata* subsp. *lusitanica* (sin. *L. pedunculata*) from S of Portugal was also dominated by fenchone and camphor but their amounts were much higher than the obtained in the present study (38.9 and 40.6 %, 41.9 and 34.6 %; respectively; Costa et al., 2013; Matos et al., 2015). The oils from the same species cultivated near Lisbon were also characterized by high fenchone content (21−59 %). However, all samples from São Domingos mine and Corte do Pinto had more borneol, cis-linalool oxide and especially, verbenone than samples analysed by Matos et al. (2015) (< 0.5 %). In the oils obtained from *L. pedunculata* collected in the North and Central Portugal, the amounts of the main compounds and even the major components were different from those determined in the plant samples from SD and CP (fenchone and 1,8-cineole or 1,8-cineole+camphor, depending on population; Zuzarte et al., 2009). Thus, amounts of each component vary widely with the population, even in non-contaminated conditions. Differences in the composition of *L. luisieri* oils extracted from plant material in different moist conditions (dry or fresh) and plant part (flowers and leaves) are reported, even if the same extraction method was used (Gomes et al., 2005; González-Coloma et al., 2011).

Significant differences were obtained among the concentrations of major compounds in *L. pedunculata* extracted in the two steps (except verbenone in 2nd step of the extraction; Table 3). However, as for single extraction, no clear tendency was obtained according to the soil contamination.

Type and amount of the compounds also varied with extraction solution being some compounds only identified in specific extract (*e.g.* Aqueous extract: coumarin, eucarvone and pinocarvone; Hexane extracts: α-pinene, aromadendrene, hinesol and tau-cadinol). In fact, solvents of different polarities extract different groups of compounds (Cowan, 1999). Besides, the extraction of some compounds, namely phenolic, in *L. pedunculata* subsp. l*usitanica* is influenced by the nature and polarity of the solvent system (Costa et al., 2013). Independently of the plant samples (except SD2 for camphene), the highest concentrations of the major compounds were obtained in the aqueous extracts, compared to the second step of the extraction with hexane (Table 3). The loss of volatile

compounds during the plant material drying after the first step of extraction can justify the decreasing of their concentrations.

Comparing the two methodologies of extraction (sequential and single with hexane), the application of the sequential extraction did not seem to have significant advantage in the recovery of more valuable compounds from *L. pedunculata*. In fact, the concentrations of the major compounds in the extracts obtained in the single extraction were significantly higher or similar to the sum of both fractions.

Taking into account the application of a single extraction, the use of water as the extracting solvent can be considered a sustainable choice compared to other alternative solvents (hexane). In fact, only the concentrations of camphene (highly volatile compounds) in hexane extract were significantly higher than those in aqueous extracts, independently of the sample. For the other quantified compounds, no clear differentiation was observed. In this way, aqueous extracts of *L. pedunculata* could be directly used as for toilet water.

Extracts of Cistus ladanifer: Component identification and quantification

In the extracts of *C. ladanifer* were identified 32 compounds, which represent between 29 and 43 % of the total compounds (Table 5). The composition of *C. ladanifer* extracts (obtained with hexane) was complex presenting a great number of compounds of difficult identification due to their very small amounts (< 0.1 %), as reported for oils of the same species (Mariotti et al., 1997; Gomes et al., 2005). Moreover, in these extracts (and especially as in aqueous extracts obtained from the same species, data not show) were found great number of undefined peaks.

Independently of the population and sample, the major component in the extracts was viridiflorol (Table 5). This compound was identified in the oils of different samples of *C. ladanifer* collected in Portugal and Morocco (14−17% and 19.4 %, respectively) being responsible for floral notes as indicated by Gomes et al. (2005) and Greche et al. (2009). On the contrary, major components in oils of other plant samples from Morocco were camphene and borneol (15.5 and 11.1 %, respectively; Zidane et al., 2013), while in oils from the same species collected in Spain were trans-pinocarveol and viridiflorol (20 and 13.6 %, respectively; Verdeguer et al., 2011). Plants from Spain cultivated in the East coast of Corsica showed, especially, α-pinene in their oils (16−47 %) but, in some samples, was also found, as major component, viridiflorol (20−23 %; Mariotti et al., 1997).

Additionally, other compounds occurred, in small amounts (< 2.5 %; Table 5), in the plant extracts from São Domingos and Corte do Pinto. These compounds have a great odoriferous interest (*e.g.* amber-like scent: ambrox and caryophyllene oxide; fresh-camphoraceous: bornyl acetate, borneol and myrtenol) (Gomes et al., 2005) and microbial effect (*e.g.* α-pinene, β-pinene, fenchone and camphor) (Chu and Kemper, 2001).

Cistus ladanifer extracts from the two studied populations exhibited similar profiles and quite variation in the qualitative composition (Table 5). These results are in agreement with those reported for other extracts (obtained with different solvents and methodologies) and populations (Gomes et al., 2005; Greche et al., 2009; Mariotti et al., 1997; Regino et al., 1987; Teixeira et al., 2007; Verdeguer et

al., 2011; Zidane et al., 2013 and references therein). Therefore, contamination in soils and potentially hazardous elements in *C. ladanifer* shoots (Fig. 1) did not affect the quality of the extracts.

Table 5 Chemical composition (%) of extracts, obtained with hexane, from *Cistus ladanifer* shoots collected in São Domingos mine (SD) and Corte do Pinto (CP; non-contaminated area) (mean ± σ; *n* = 3).

LRI	Compound identified	SD3	SD4	SD5	CP1
902	α-Thujene	0.10 ± 0.02	0.23 ± 0.07	0.09 ± 0.03	0.22 ± 0.01
916	α-Pinene	1.31 ± 0.26	2.02 ± 0.48	2.35 ± 0.80	2.16 ± 0.52
935	Camphene	0.41 ± 0.11	1.00 ± 0.25	0.32 ± 0.08	0.92 ± 0.05
940	β-Pinene	0.25 ± 0.05	0.26 ± 0.06	0.35 ± 0.09	0.46 ± 0.10
1020	1,3,8-p-Menthatriene	0.20 ± 0.03	0.22 ± 0.02	0.28 ± 0.09	0.32 ± 0.01
1030	2,2,6-Trimethylcyclohexanone	1.46 ± 0.43	0.75 ± 0.06	1.26 ± 0.56	2.26 ± 0.18
1068	Linalool oxide cis	0.20 ± 0.03	0.20 ± 0.01	0.21 ± 0.07	0.29 ± 0.03
1082	Fenchone	0.26 ± 0.05	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
1103	2,4-Dimethylcyclohexanol	1.04 ± 0.18	0.70 ± 0.10	0.96 ± 0.31	1.49 ± 0.15
1121	6-Camphenol	0.15 ± 0.01	0.18 ± 0.03	0.19 ± 0.06	0.22 ± 0.00
1134	Isopinocarveol	0.33 ± 0.05	0.44 ± 0.06	0.38 ± 0.11	0.47 ± 0.07
1139	Camphor	0.81 ± 0.14	0.37 ± 0.04	0.34 ± 0.13	0.41 ± 0.05
1142	cis-Verbenol	0.42 ± 0.10	0.63 ± 0.06	0.79 ± 0.27	0.72 ± 0.15
1156	Pinocarvone	0.11 ± 0.02	0.11 ± 0.03	0.12 ± 0.03	0.12 ± 0.00
1166	Borneol	0.85 ± 0.16	1.18 ± 0.13	0.84 ± 0.28	1.46 ± 0.16
1176	β-Terpineol	0.20 ± 0.06	0.19 ± 0.03	0.28 ± 0.10	0.37 ± 0.06
1182	p-Cymen-8-ol	0.40 ± 0.06	0.44 ± 0.03	0.51 ± 0.21	0.74 ± 0.01
1190	Myrtenol	0.27 ± 0.07	0.50 ± 0.10	0.40 ± 0.16	0.43 ± 0.00
1200	Verbenone	2.21 ± 0.28	2.34 ± 0.28	2.75 ± 0.83	3.56 ± 0.25
1217	trans-Carveol	0.58 ± 0.54	0.37 ± 0.07	0.45 ± 0.09	0.51 ± 0.09
1280	Bornyl acetate	1.07 ± 0.22	1.37 ± 0.17	0.97 ± 0.32	1.54 ± 0.13
1298	Thymol	0.52 ± 0.12	0.45 ± 0.02	0.73 ± 0.35	1.12 ± 0.24
1347	Benzenepropanoic acid	0.65 ± 0.40	0.74 ± 0.33	0.59 ± 0.59	2.45 ± 1.96
1547	Frambinone	0.61 ± 0.13	0.74 ± 0.06	1.35 ± 0.61	0.69 ± 0.10
1561	Rhododendrol	0.76 ± 0.26	1.17 ± 0.27	2.51 ± 0.87	1.15 ± 0.08
1572	Spathulenol	0.30 ± 0.07	0.46 ± 0.02	0.41 ± 0.09	0.24 ± 0.03
1587	Viridiflorol	8.86 ± 1.76	9.09 ± 1.12	12.23 ± 5.50	12.81 ± 2.01
1596	Globulol	2.04 ± 0.42	2.26 ± 0.35	2.88 ± 1.25	2.54 ± 0.29
1601	Caryophyllene oxide	0.93 ± 0.21	1.24 ± 0.13	1.03 ± 0.57	1.59 ± 0.45
1602	Cubenol	0.66 ± 0.39	0.98 ± 0.03	0.87 ± 0.41	1.23 ± 0.46
1748	n-Heptadecene	0.36 ± 0.09	0.11 ± 0.02	0.38 ± 0.12	0.39 ± 0.10
1753	Ambrox	0.15 ± 0.07	0.12 ± 0.05	0.15 ± 0.05	0.12 ± 0.03
Total identified compounds		**29.89 ± 6.49**	**37.73 ± 14.99**	**31.50 ± 3.51**	**44.82 ± 6.25**

LRI: Linear retention indices determined with homologous series of n-alkanes

A wide variation in the composition of this species extracts can be explained by the analysed plant part and its conditions of collection, drying and storage as well as method of extraction (Raut and Karuppayil, 2014). In fact, Gomes et al. (2005) found that the use of dry material, instead of fresh material, can affect the amounts of the components with middle to high volatility in *C. ladanifer* oils, however the influence level seems to depend on the population (located N or S of Portugal). Composition and yield of *C. ladanifer* oil also vary along the year, storage time and with the different geographical sources of the plants, as well as the solvent used in the extraction (Regino et al., 1987). The influence of the solvent and/or the analysed part of the plant (flowers, fruit, stem or leaves) on the extracted content of flavonoids and phenolics from *C. ladanifer* shoots is reported (Andrade et al., 2009; Zidane et al., 2013). Even using another methodology of extraction (simultaneous distillation–extraction) with several solvents (pentane, cyclohexane, hexane and diethyl, ether and mixture of pentane/diethyl ether), a distinct efficiency of extraction was observed according on compound. However, in general, the average extraction efficiencies were quite similar (Teixeira et al., 2007).

Concerning the concentrations of some major components (Table 6), some significant differences were obtained among samples. Sample SD3 had more camphor and fenchone while in SD4 was determined the highest camphene concentrations. For α-pinene and verbenone, some samples from the mining area showed similar concentrations compared to the samples from non-contaminated area (CP1). However, no correlation was obtained between concentrations of the major components and the potentially hazardous elements in shoots. Thus, inter or intra-variability of the plants and their conditions of growth and/or ecological stress do not justified these variations. Possibly, the type of plant material used, *i.e*. composite samples of shoots, which may include different proportions of leaves, stems and flowers, can contribute to the variation of the compounds concentrations.

Table 6 Concentration of some major components (mg/kg) in extracts, obtained with hexane, from *Cistus ladanifer* shoots collected in São Domingos mine (SD) and Corte do Pinto (CP; non-contaminated area) (mean ± σ; *n* = 3).

			mg/kg		
Samples	**α-Pinene**	**Camphene**	**Camphor**	**Fenchone**	**Verbenone**
SD3	114.9 ± 29.3^{ab}	29.9 ± 7.0^{bc}	70.2 ± 4.1^{a}	20.7 ± 0.2^{a}	232.0 ± 6.9^{a}
SD4	124.5 ± 12.4^{ab}	53.3 ± 5.0^{a}	31.3 ± 5.0^{b}	4.5 ± 0.9^{b}	131.8 ± 12.6^{b}
SD5	163.7 ± 43.8^{a}	20.8 ± 5.5^{c}	39.1 ± 13.6^{b}	5.4 ± 1.3^{b}	200.9 ± 85.1^{ab}
CP1	107.8 ± 3.9^{b}	39.6 ± 8.3^{b}	14.5 ± 0.2^{c}	4.2 ± 1.9^{b}	189.9 ± 33.6^{ab}

Values for each compound followed by a different letter are significantly different ($p < 0.05$)

CONCLUSION

Soils from São Domingos can be considered contaminated with As, Cu, Pb and Sb. The concentrations of the potentially hazardous elements in the shoots of *C. ladanifer* and *L. pedunculata* from São Domingos presented intrapopulation variability in their concentrations of the potentially hazardous elements. In general, concentrations of the studied elements in shoots of both species

(excepted Cr and Mn in *L. pedunculata* and Cr in *C. ladanifer*) collected in São Domingos mining area were higher than in the same species from the non-contaminated area.

The infusions done with *L. pedunculata* shoots from São Domingos had only concentrations of Zn significantly different compared to the infusions of the plants from the non-contaminated area. Independently of the population concentrations of the potentially hazardous elements in *L. pedunculata* infusions were small so, this product can be considered for human consumption without any risk. Extracts obtained from *C. ladanifer* and *L. pedunculata* growing in São Domingos mining area had valuable compounds which might be used for a wide range of applications. Since the extracts are complex mixtures of different compounds, it is difficult to attribute their biological/odoriferous activity to a single or a particular constituent. However, extracts of both species presented great potential pharmaceutical and fragrance/perfumery industry.

Qualitative composition of the extracts was almost constant over the range of concentrations of the elements in the soils (contaminated and non-contaminated). This study demonstrated that high concentrations of potentially hazardous elements in soils did not affect the quality of the extracts. In this way, phytostabilisation of mining areas using these species can provide economic return by the exploration of these products. However, it is essential to confirm the concentration of hazardous elements in the extracted plant residues. In order to improve the efficiency, the extraction should be adjusted by setting the operation conditions pressure and temperature.

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REFERENCES

Abreu, M.M., Magalhães, M.C.F., 2009. Phytostabilization of soils in mining areas. Case studies from Portugal. In: Aachen, L., Eichmann, P. (Eds.), Soil remediation. Nova Science Publishers, Inc. New York, pp. 297-344.

Abreu, M.M., Tavares, M.T., Batista, M.J., 2008. Potential use of *Erica andevalensis* and *Erica australis* in phytoremediation of sulphide mine environments: São Domingos, Portugal. Journal of Geochemical Exploration 96(2-3), 210-222.

Abreu, M.M., Santos, E.S., Ferreira, M., Magalhães, M.C.F., 2012a. *Cistus salviifolius* a promising species for mine wastes remediation. Journal of Geochemical Exploration 113, 86-93.

Abreu, M.M., Santos, E.S., Fernandes, E., Magalhães, M.C.F., 2012b. Trace elements tolerance, accumulation and translocation in *Cistus populifolius*, *Cistus salviifolius* and their hybrid growing in polymetallic contaminated mine areas. Journal of Geochemical Exploration 123, 52-60.

Activation Laboratories, 2015a. Code 1H – Au + 48. Available in: http://www.actlabs.com/page.aspx?page=506&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes. Accessed May 2015.

Activation Laboratories, 2015b. 2D - Vegetation Ash - ICP/MS. Available in: http://www.actlabs.com/page.aspx?page=538&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes. Accessed May 2015.

Altıntıg, E., Altundag, H., Tuzen, M., 2014. Determination of multielement levels in leaves and herbal teas from Turkey by ICP-OES. Bulletin of the Chemical Society of Ethiopia 28(1), 9-16.

Álvarez-Valero, A.M., Pérez-López, R., Matos, J., Capitán, M.A., Nieto, J.M., Sáez, R., Delgado, J., Caraballo, M., 2008. Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian Peninsula): evidence from a chemical and mineralogical characterization. Environmental Geology 55(8), 1797-1809.

Anawar, H.M., Freitas, M.C., Canha, N., Regina, I.S., 2011. Arsenic, antimony, and other trace element contamination in a mine tailings affected area and uptake by tolerant plant species. Environmental Geochemistry and Health 33, 353-362.

Anawar, H.M., Canha, N., Santa-Regina, I., Freitas, M.C. 2013. Adaptation, tolerance, and evolution of plant species in a pyrite mine in response to contamination level and properties of mine tailings: sustainable rehabilitation. Journal of Soils and Sediments 13, 730-741.

Andrade, D., Gil, C., Breitenfeld, L., Domingues, F., Duarte, A.P., 2009. Bioactive extracts from *Cistus ladanifer* and *Arbutus unedo* L.. Industrial Crops and Products 30, 165-167.

Attaguile, L.G., Russo, A., Campisi, A., Savoca, F., Acquaviva, R., Ragusa, N., Vanella, A., 2000. Antioxidant activity and protective effect on DNA cleavage of extracts from *Cistus incanus* L. and *Cistus monspeliensis* L.. Cell Biology and Toxicology 16,83-90.

Barros, L., Dueñas, M., Alves, C.T., Silva, S., Henriques, M., Santos-Buelga C., Ferreira, I.C.F.R., 2013. Antifungal activity and detailed chemical characterization of *Cistus ladanifer* phenolic extracts. Industrial Crops and Products 41, 41-45.

Cavanagh, H.M.A., Wilkinson, J.M., 2002. Biological activities of lavender essential oil. Phytotherapy Research 16, 301-308.

CCME – Canada Council of Ministers of the Environment, 2007. Canadian soil quality guidelines for the protection of environmental and human health: Summary tables (updated September, 2007). CCME, Winnipeg, Canada.

Chu, K.J., Kemper. Lavender (*Lavandula* spp.). Available in: http://www. mcp.edu/herbal (accessed November 2015).

Costa, P., Gonçalves, S., Valentão, P., Andrade, P.B., Almeida, C., Nogueira, J.M.F., Romano, A., 2013. Metabolic profile and biological activities of *Lavandula pedunculata* subsp. *lusitanica* (Chaytor) Franco: Studies on the essential oil and polar extracts. Food Chemistry 141, 2501-2506.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews 12, 564-582.

Danh, L.T., Triet, N.D.A., Han, L.T.N., Zhao, J., Mammucari, R., Fostera, N., 2012. Antioxidant activity, yield and chemical composition of lavender essential oil extracted by supercritical CO_2. Journal of Supercritical Fluids 70, 27-34.

de la Fuente, V., Rufo, L., Rodríguez, N., Amils, R., Zuluaga, J., 2010. Metal accumulation screening of the Río Tinto Flora (Huelva, Spain). Biological Trace Element Research 134, 318-341.

Durães, N., Bobos, I., Ferreira da Silva, E., Dekayir, A., 2015. Copper, zinc and lead biogeochemistry in aquatic and land plants from the Iberian Pyrite Belt (Portugal) and north of Morocco mining areas. Environmental Science and Pollution Research 22, 2087-2105.

Elaissi, A., Rouis, Z., Salem, N.A.B., Mabrouk, S., Salem, Y.B., Salah, K.B.H., Aouni, M., Farhat, F., Chemli, R., Harzallah-Skhiri, F., Khouja, M.L., 2012. Chemical composition of 8 eucalyptus species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. BMC Complementary and Alternative Medicine 12(81), 15 pp.

Figueiredo, A.C., Pedro, L.G., Barroso, J.G., Trindade, H., Sanches, J., Oliveira, C., Correia, M., 2014. *Lavandula luisieri* (Rozeira) Rivas-Martínez e *Lavandula pedunculata* (Mill.) Cav.. Revista Agrotec 14, 38-41.

Freitas, H., Prasad, M.N.V., Pratas, J., 2004. Plant community tolerant to trace elements growing on degraded soils of São Domingos mine in the south east of Portugal: environmental implications. Environment International 30, 65-72.

Freitas, M.C., Pacheco, A.M.G., Anawar, H.M., Dionísio, I., Dung, H.M., Canha, N., Bettencourt, A., Henriques, F., Pinto-Gomes, C.J., Capelo, S., 2009. Determination of phytoextraction potential of plant species for toxic elements in soils of abandoned sulphide-mining areas. Journal of Radioanalytical and Nuclear Chemistry 282, 21-27.

Gomes, P.B., Mata, V.G., Rodrigues, A.E., 2005. Characterization of the Portuguese-grown *Cistus ladanifer* essential oil. Journal of Essential Oil Research 17, 160-165.

González-Coloma, A., Martín-Benito, D., Mohamed, N., García-Vallejo, M.C., Soria, A.C., 2006. Antifeedant effects and chemical composition of essential oils from different populations of *Lavandula luisieri* L.. Biochemical Systematics and Ecology 34, 609-616.

Gonçalves, M., Santos, E., Duarte, I., Marques, D., Mendes, B., 2013. Composition and functional properties of essential oils extracted from forest waste biomass. STUDIA – Scientiæ Rerum Diffusio: Suplemento Temático IV Seminário Luso-Brasileiro em Ciências do Ambiente e Empresariais, 4 pp.

Görür, F.K., Keser, R., Akçay, N., Dizman, S., Okumuşoğlu, N.T., 2011. Radionuclides and heavy metals concentrations in Turkish market tea. Food Control 22, 2065-2070.

Greche, H., Mrabet, N., Zrira, S., Ismaili-Alaoui, M., Benjilali, B., Boukir, A., 2009. The volatiles of the leaf oil of *Cistus ladanifer* L. var. *albiflorus* and labdanum extracts of Moroccan origin and their antimicrobial activities. Journal of Essential Oil Research 21(2), 166-173.

IUSS Working Group WRB, 2007. World Reference Base for Soil Resources 2006 (first update 2007). World Soil Resources Reports No. 103, Rome, FAO.

Mariotti, J.P., Tomi, F., Casanova, J., Costa, J., Bernardini, A.F., 1997. Composition of the essential oil of *Cistus ladaniferus* L. cultivated in Corsica (France). Flavour and Fragrance Journal 12(3), 147-151.

Matos, J.X., Martins, L.P., 2006. Reabilitação ambiental de áreas mineiras do sector português da Faixa Piritosa Ibérica: estado da arte e perspectivas futuras. Boletín Geológico y Minero 117, 289-304.

Matos, F., Miguel, M.G., Duarte, J., Venâncio, F., Moiteiro, C., Correia, A.I.D., Figueiredo, A.C., Barroso, J.G., Pedro, L.G., 2009. Antioxidant capacity of the essential oils from *Lavandula luisieri*, *L. stoechas* subsp. *lusitanica*, *L. stoechas* subsp. *lusitanica* x *L. luisieri* and *L. viridis* grown in Algarve (Portugal). Journal of Essential Oil Research 21(4), 327-336.

Mendez, M.O., Maier, R.M., 2008. Phytoremediation of mine tailings in temperate and arid environments. Reviews in Environmental Science and Biotechnology 7, 47-59.

Pérez-López, R., Álvarez-Valero, A.M., Nieto, J.M., Sáez, R., Matos, J.X., 2008. Use of sequential extraction procedure for assessing the environmental impact at regional scale of the São Domingos Mine (Iberian Pyrite Belt). Applied Geochemistry 23, 3452-3463.

Pérez-López, R., Márquez-García, B., Abreu, M.M., Nieto, J.M., Córdoba, F., 2014. *Erica andevalensis* and *Erica australis* growing in the same extreme environments: Phytostabilization potential of mining areas. Geoderma 0-231, 194-203.

Phytochemical and ethnobotanical databases, 2015. Dr. Duke's Phytochemical and Ethnobotanical Databases. Available in: http://www.ars-grin.gov/duke/ (accessed November 2015).

Póvoas, I., Barral, M.F., 1992. Métodos de análise de solos. Comunicações do Instituto de Investigação Científica Tropical. Serie Ciências Agrárias 10, Lisboa.

Quental, L., Bourguignon, A., Sousa, A.J., Batista, M.J., Brito, M.G., Tavares, T., Abreu, M.M., Vairinho, M., Cottard, F., 2002. MINEO Southern Europe environment test site. Contamination impact mapping and modelling − Final Report. Assessing and monitoring the environmental impact of mining activities in Europe using advanced earth observation techniques (MINEO) 5yth FP-IST-1999-10337.

Raut, J.S., Karuppayil, M., 2014. A status review on the medicinal properties of essential oils. Industrial Crops and Products 62, 250-264.

Regino, J.M.B., Frazão, S., Carmo, M.M., Venâncio, F., 1987. Estudo da partevolátil do concreto de esteva (*Cistus ladanifer* L.) em comparação com oóleo essencial. 2as. Jornadas Nacionais de Plantas Aromáticas e Óleos Essenciais, Lisboa.

Santos, E.S., Abreu, M.M., Nabais, C., Saraiva, J., 2009. Trace elements and activity of antioxidative enzymes in *Cistus ladanifer* L. growing on an abandoned mine area. Ecotoxicology 18, 860-868.

Santos, E.S., Abreu, M.M., Nabais, C., Magalhães, M.C.F., 2012. Trace element distribution in soils developed on *gossan* mine wastes and *Cistus ladanifer* L. tolerance and bioaccumulation. Journal of Geochemical Exploration 123, 45-51.

Santos, E.S., Abreu, M.M., Batista, M.J., Magalhães, M.C.F., Fernandes, E., 2014. Inter-population variation on the accumulation and translocation of potentially harmful chemical elements in *Cistus ladanifer* L. from Brancanes, Caveira, Chança, Lousal, Neves Corvo and São Domingos mines in the Portuguese Iberian Pyrite Belt. Journal of Soils and Sediments 14, 758-772.

Tavares, M.T., Sousa, A.J., Abreu, M.M., 2008. Ordinary kriging and indicator kriging in the cartography of trace elements contamination in São Domingos mining site (Alentejo, Portugal). Journal of Geochemical Exploration 98, 43-56.

Teixeira, S., Mendes, A., Alves, A., Santos, L., 2007. Simultaneous distillation-extraction of high volatile compounds from *Cistus ladanifer* L.. Analytica Chimica Acta 584, 439-446.

Verdeguer, M., Blázquez, M.A., Boira, H., 2011. Chemical composition and herbicidal activity of the essential oil from a *Cistus ladanifer* L. population from Spain. Natural Product Research, 6(17), 1602-1609.

Wong, M.H., 2003. Ecological restoration of mine degraded soils with emphasis on metal contaminated soils. Chemosphere 50, 775-780.

Zheljazkov, V.D., Nielsen, N.E., 1996. Studies on the effect of heavy metals (Cd, Pb, Cu, Mn, Zn and Fe) upon the growth, productivity and quality of Lavender (*Lavandula angustifolia* Mill.). Production Journal of Essential Oil Research 8(3), 259-274.

Zheljazkov, V.D., Craker, L.E., Xing, B., 2006. Effects of Cd, Pb, and Cu on growth and essential oil contents in dill, peppermint, and basil. Environmental and Experimental Botany 58, 9-16.

Zheljazkov, V.D., Craker, L.E., Xing, B., Nielsen, N.E., Wilcox, A., 2008. Aromatic plant production on metal contaminated soils. Science of the Total Environment 395, 51-62.

Zidane, H., Elmiz, M., Aouinti, F., Tahani, A., Wathelet, J., Sindic, M., Elbachiri, A., 2013. Chemical composition and antioxidant activity of essential oil, various organic extracts of *Cistus ladanifer* and *Cistus libanotis* growing in Eastern Morocco. African Journal of Biotechnology 12(34), 5314-5320.

Zuzarte, M., Gonçalves, M.J., Cavaleiro, C., Dinis, A.M., Canhoto, J.M., Salgueiro, L.R., 2009. Chemical composition and antifungal activity of the essential oils of *Lavandula pedunculata* (Miller) Cav.. Chemistry & Biodiversity 6, 1283-1292.

6. POTENTIAL ENVIRONMENTAL IMPACT OF TECHNOSOLS COMPOSED OF GOSSAN AND SULFIDE-RICH WASTES FROM SÃO DOMINGOS MINE: ASSAY OF SIMULATED LEACHING

ABSTRACT

This study aimed to evaluate: the effectiveness of two Technosols prepared with *gossan* or sulfide-rich wastes and organic/inorganic amendment mixtures, by the analysis of the over time variation of the elemental concentrations in their simulated leachates; and the potential environmental risk of these Technosols evaluated from the mass of the leached potentially hazardous elements calculated taking into account the leachates elemental concentrations and the tailings mass in São Domingos mine area.

Composite samples of two São Domingos mining wastes (*gossan* wastes − GW; sulfide-rich wastes − SW) were collected. Amendment mixtures, containing different organic/inorganic wastes (from green-agriculture, distillation of *Ceratonia siliqua* L. and *Arbutus unedo* L. fruits and limestone quarry), were applied at 12, 30 and 60 g/kg to GW and SW. Two sets of microcosm pot assays were performed under controlled conditions in greenhouse, and monitored during seven and thirteen months for GW and SW, respectively. Materials from each pot/treatment (< 5 cm of depth) were collected after one, four, seven and thirteen months of incubation, and used to obtain simulated leachates (DIN extraction). The leachates were analysed for pH, electrical conductivity, and the concentrations of anions and cations by F-AAS, GF-AAS, HPLC and UV-VIS.

Simulated leachates from SW had lower pH and higher concentrations of potentially hazardous elements than leachates from GW. The concentrations of As in leachates from GW-Technosols were higher than those in leachates from GW-control but < 0.6 mg/kg. In GW-Technosols leachates, the pH and the concentrations of some nutrients (*e.g.* Ca and phosphates) also increased when compared to control. In the SW-Technosols, the pH of the leachates increased only in the first month as long as limestone was present. In these leachates was observed a general trend of concentrations decrease for some elements (*e.g.* As, sulfate, Fe, Pb), especially in the first month. A clear influence of the dose and type of amendments was not observed during the experimental time span for both wastes.

The concentrations of the elements like Al, Ca, Fe and Pb in the leachates are controlled by both the pH of the solutions and the concentrations of phosphates and sulfates. The extensive mass of the studied mine wastes contribute to the release to the neighbouring environment of considerable amounts of potentially hazardous elements. The rehabilitation of the mine wastes by the conception of Technosols, especially with sulfide-rich wastes, can reduce significantly the environmental impact.

KEYWORDS DIN extraction • Environmental impact • Potentially hazardous elements • Leaching methods

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INTRODUCTION

In the Iberian Pyrite Belt (IPB), one of the most important metallogenic provinces of massive sulfides in the world, several mining areas are abandoned generating great environmental problems associated to sulfide wastes (Abreu et al., 2010; Matos and Martins, 2006; Sánchez-España et al., 2005). The oxidation of the sulfides produces extreme acid mine drainage (AMD) containing high concentrations of potentially hazardous elements (Abreu et al., 2010; Sánchez-España et al., 2005), which can be spread to the adjacent areas.

São Domingos mine (SE of Portugal) is one of the abandoned mines located in the Portuguese IPB that present severe environmental impacts due to the high volume of spoils and tailings and the dimension of the areas affected by AMD (Álvarez-Valero et al., 2008; Matos and Martins, 2006). The exploitation and extraction processes in this mine (between pre-roman period and 1966) produced between 27 and 32 Tg of different mining wastes, which include around 17 % of sulfide-rich wastes (*e.g.* pyrite blocks, brittle pyrite, modern and Roman slags and smelting ashes) and 15 % of *gossan* wastes (Álvarez-Valero et al., 2008; Pérez-López et al., 2008). The sulfides-rich tailings, mainly composed of crushed pyrite and smelting ashes, show a very low pH and a large capacity to generate, continuously, AMD (Santos et al., 2014a). However, both mine wastes have high total concentrations of several elements contributing to a multielemental contamination through their leachates (Abreu et al., 2008, 2010; Álvarez-Valero et al., 2008; Pérez-López et al., 2008; Santos et al., 2014a, 2015).

The ecological rehabilitation of the São Domingos mining area should be initiated by the improvement of the chemical characteristics of mine wastes, especially sulfide-rich wastes, in order to minimize the leaching and/or elements concentrations in the leachates. These waste materials are bared and subject to more or less intense water erosion. The tailings, mainly composed of *gossan* materials, are naturally colonized by vegetation and present low reactivity and capacity to generate AMD. However, this vegetation is sparse and their growth is quite slow. In order to stimulate the *in situ* plant growth, a more effective plant cover and species diversity, as well as the substratum microbial activity related to nutrients cycling, the physical and chemical characteristics (especially fertility) of the wastes should be improved.

The application of amendment mixtures to the mine wastes, which makes a Technosol, can be a valuable technique because these amendments can decrease the potentially hazardous elements concentrations in the leachates, can create/promote the different biological soil functions, improving the wastes structure and water holding capacity and, consequently, contributing to the rehabilitation of the area (Kumpiene et al., 2008; Macías 2004; Macías et al., 2011).

Some studies discuss the potential hazard of mine wastes (amended or not) based on the elemental concentrations in the available fraction (Adriano et al., 2004; Forsberg et al., 2008; Reis et al., 2012; Santos et al., 2014a) and/or ecotoxicological indicators (Alvarenga et al., 2009; González et al., 2012; Santos et al., 2013, 2015). To the best of our knowledge, there is no information concerning the amount of potentially hazardous elements that can be released to the surrounding environment, by leaching, from the entire mine wastes mass present in the field, mass when a rehabilitation process is

to be implemented. In order to optimize a Technosol, it is crucial to measure the chemical elements concentrations in the leachates.

Several leaching tests are used for scientific purposes, in an attempt to simulate the leaching processes at which the materials are exposed in the field during the rain events in order to evaluate the leachates quality and the elements concentrations leached from the materials (Chezom et al., 2013; Fällman and Aurell, 1996; Perera et al., 2005).

The DIN method (DIN 38414-S4 extraction), one of the most commonly used, is a standard batch test widely used for solid wastes and recommended by European Union (Kocasoy and Murat, 2009). It has been regulatory compliance in Germany and Austria, and was used as basis for the European standard leaching method EN 12457 and other tests recently developed under CEN TC/292 (Perera et al., 2005). In this method, is used a 1:10 (*m*:*V*) solid wastes:distilled (deionized) water ratio, continuously agitated for 24 h. The water percolation through the waste materials is also used as a direct and simple percolation method of obtaining leachates (Pérez-López et al., 2008; Santos et al., 2014a, 2015; Yao et al., 2008).

This study aimed to evaluate: i) the variations of the elemental concentrations in the simulated leachates from Technosols, prepared with *gossan* or sulfide-rich mine wastes and organic/inorganic amendments after one, four, seven and thirteen months of incubation; and ii) the potential environmental risk of these Technosols evaluated from the mass of the leached potentially hazardous elements calculated taking into account the leachates elemental concentrations and the tailings mass in São Domingos mining area.

MATERIALS AND METHODS

Components of the Technosols

Composite samples of two mining wastes (*gossan* wastes – GW and sulfide-rich wastes – SW) were collected within São Domingos mine in 2009, close to the open pit (GW) and in the south-west zone of the open pit near the former copper leaching tanks (SW). These mine wastes were mainly chosen owing to their great volumetry and wide distribution in São Domingos mine, as well as their chemical characteristics (Álvarez-Valero et al., 2008; Quental et al., 2002, 2013).

The Technosols were composed of *gossan* (GW) or sulfide-rich wastes (SW) and amendment mixtures containing distinct organic/inorganic wastes from green-agriculture (plant remains + strawberry substrate at 2:3 *m*/*m* – AW; and rockwool used for strawberry crops – RW) and small distilleries (*Arbutus unedo* L. and *Ceratonia siliqua* L. fruit spirits distillation – AuW and CW, respectively) at 12 g/kg, 30 g/kg and 60 g/kg of each mine waste. In Technosols containing SW were also applied residues from quarry (limestone rock wastes – LW), at 22 g/kg. Seven Technosols were tested for each mine waste (*n* = 3 or 4 for each Technosol made with GW or SW, respectively) (Table 1). In order to assess the efficiency of the Technosols, control treatments were also considered containing only the mine wastes (*n* = 3 or 4 for GW or SW, respectively).

The organic and inorganic amendments were used due to their large quantities in the mine vicinity, very small cost (only associated to the transport) and beneficial and safe characteristics. The chemical characteristics of the used materials are given in Table 2.

Experimental set up and samples analysis

The mine wastes and amendments were air-dried. All materials were mixed manually, potted and maintained at 70 % of their water-holding capacity. Two sets of microcosm assays were performed in pots (≈2 kg of each mine waste; Assay 1 – total fraction of SW and Assay 2 – fraction < 10 mm of GW) under controlled conditions in greenhouse. The assays were monitored during seven and thirteen months for GW (n = 3 per treatment) and SW (n = 4 per treatment), respectively.

Samples from control (GW or SW) and Technosols were collected (< 5 cm depth) after one, four, seven and thirteen months of incubation. These materials were homogenized, sieved (< 2 mm) and kept fresh (4 ºC) until chemical analysis. They were used to obtain simulated leachates (DIN 38414-S4 extraction). In the method DIN, the different chemical elements were extracted from the solid phases by contact with distilled water (1:10 m/V), for 24 h in a rotatory agitator. Following the DIN extraction, pH and electrical conductivity (EC) were measured after 15 min and 24 h of agitation. Simulated leachates were filtrated (0.45 µm) and stored at –18 ºC until the analysis. In the leachates were determined the concentrations of: Al, Ca, Cu, Fe, K, Mg, Mn, Pb and Zn by flame atomic absorption spectrometry; As by graphite furnace atomic absorption spectrometry; chlorides, nitrates and sulfates by ion chromatography; and phosphates by visible spectrophotometry (molybdenum blue method; USEPA 1979).

Data analysis

Statistical analysis was performed with the statistical programme SPSS v18.0 for Windows. Data were analysed by one way ANOVA, and post-hoc Duncan's test (p < 0.05) was used to separate means. Data not satisfying assumptions for ANOVA were analysed non-parametrically using Kruskal-Wallis ANOVA by a rank test. For statistical purposes, the results below the detection limit were assumed as half of the detection limit.

The potential environmental risk of SW, GW, and respective Technosols was evaluated from the mass of the leached potentially hazardous elements. For the calculations were used the minimum and maximum concentration of the potentially hazardous elements in the simulated leachates, and the mass of tailings in São Domingos mining area reported by Álvarez-Valero et al. (2008) and Pérez-López et al. (2008).

Table 1 Composition of the Technosols used for the leaching assays.

Mine waste	Amendment mixture	Rate application (g/kg of mine waste)	Other inorganic wastes	Rate of application (g/kg of mine waste)	Technosols designation
Gossan wastes (GW) *Assay 1*	AW + AuW + RW	12, 30; 60	---	---	G-A12; G-A30; G-A60
	AW + CW + RW	12; 30	---	---	G-B12; G-B30
	AW + AuW + CW + RW	12; 30	---	---	G-C12; G-C30
Sulfide-rich wastes (SW) *Assay 2*	AW + AuW + RW	12, 30; 60	LW	22	S-A12; S-A30; S-A60
	AW + CW + RW	12; 30	LW	22	S-B12; S-B30
	AW + AuW + CW + RW	12; 30	LW	22	S-C12; S-C30

AW: Agriculture wastes; AuW: residue from the liquor distillation of *Arbutus unedo* L. fruit; CW: residue from liquor distillation of *Ceratonia siliqua* L. fruit; RW: Rockwool used for strawberry crops; LW: limestone rock wastes

Table 2 Chemical characteristics of the *gossan* and the sulfide-rich wastes from the São Domingos mine area and organic/inorganic wastes used as amendments (min – max or mean value) (Santos et al. 2014a,b).

	GW	SW	AW	AuW	CW	RW
pH (H_2O)	4.32	2.1 – 2.3	6.6 – 7.2	4.9	6.1	7.1
Electrical conductivity (mS/cm)	<0.1	7.4 – 7.5	0.9 – 3.5	1.7	0.2	3.5
Organic C (g/kg)	12.0	243.8 – 276.1*	286.1	442.6	436.7	111.0
Total N (g/kg)	0.04	3.7 – 4.8	9.7	8.9	11.1	9.8
Extractable P (g/kg)	1.3×10^{-3}	< DL	0.3 – 3.1	0.2	0.1	7.9
Extractable K (g/kg)	0.08	0.02 – 0.06	0.2 – 6.6	3.6	9.1	0.7
	Total concentrations (g/kg dry weight)					
Al	24.8	54.8 – 61.2	4.06	1.37	0.6×10^{-3}	53.4
As	3.03	1.02 – 1.14	2.3×10^{-3}	1.0×10^{-3}	0.8×10^{-3}	$< 0.5 \times 10^{-3}$
Ca	0.5	6.40 – 6.70	22.56	10.30	11.02	139.0
Cu	0.23	1.84 – 2.45	44.6×10^{-3}	0.12	9.4×10^{-3}	0.08
Fe	129	104 – 110	2.96	3.30	0.99	39.90
K	21.2	12.8 – 14.3	1.495	> 1.32	0.96	6.80
Mg	1.2	1.4 – 1.8	2.34	1.13	> 9.63	42.10
Mn	62.0×10^{-3}	0.12 – 0.16	0.25	51.2×10^{-3}	43.1×10^{-3}	1.85
Na	1.7	4.20 – 4.60	0.31	0.40	0.40	13.10
Pb	1.08	8.97 – 13.80	5.0×10^{-3}	3.1×10^{-3}	0.9×10^{-3}	$< 3 \times 10^{-3}$
S	9.21	63.6 – 68.0	NA	NA	NA	2.60
Zn	13.7	1.08 – 1.19	75.6×10^{-3}	27.3×10^{-3}	18.5×10^{-3}	0.20

*Total C concentration; AW: Agriculture wastes; AuW: residue from the liquor distillation of *Arbutus unedo* L. fruit; CW: residue from liquor distillation of *Ceratonia siliqua* L. fruit; RW: Rockwool used for strawberry crops; DL: detection limit; NA: non analysed

RESULTS AND DISCUSSION

Technosols composed of sulfide-rich wastes and amendment mixtures

Leachates electrical conductivity and pH

The pH of the leachates from sulfide-rich wastes (SW) was very low (2.1−2.4, Fig. 1B) and the EC reached values ranging between 2.9 and 5.2 mS/cm depending on sampling period (Fig. 1D). These results were in the same range than acid mine drainages from São Domingos mine and other mines belonging to the IPB (Abreu et al., 2010; Sanchez-España et al., 2005, 2008). Nevertheless, the pH values of leachates from the present study were lower than those in simulated leaching (1:20 *m*:*V*) using mineralized rock samples from Furtei gold mine (2.6−5.0; Da Pelo et al., 2009). The pH values measured in the sulfide-rich wastes from São Domingos can be related to their content in sulfides.

After one month of incubation, the pH of the leachates from SW-Technosols increased to values between 3.8 and 5.1 (Fig. 1B) due to the presence limestone rock wastes. However, after four months, the pH of the leachates of these SW-Technosols decreased to the initial value, similar to the leachates from control (pH ≈2.4), due to the continuous generation of acid drainage, which promoted the carbonates dissolution. In fact, the variation of pH between 15 min and 24 h confirm that limestone was not present in SW-Technosols (Figs. 4A and 4B) after the fourth month of incubation.

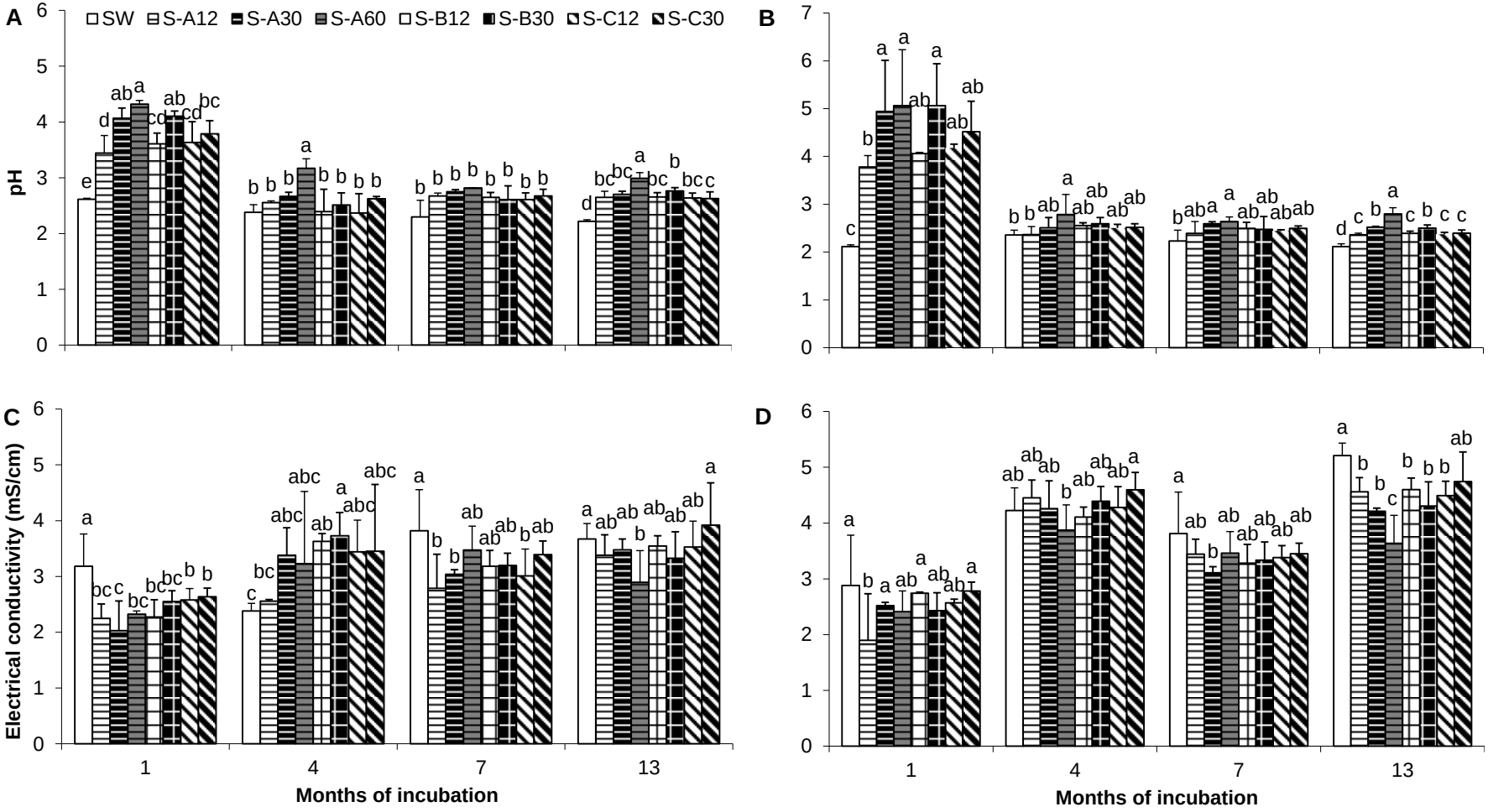
Although the pH values have remained without any further change until the end of the experiment, the leachates from SW-Technosols had pH values slightly higher than control leachates (especially at the end of the experiment where leachates pH of all SW-Technosols was significantly higher than control; Fig. 1B). This small increase in the pH can be related to the action of organic acids from amendments which have some buffering capacity (data not shown) and/or inhibition of the sulfide minerals oxidation by the organic matter.

During the experiment, some SW-Technosols showed a decrease of EC in leachates when compared to the control (except in the fourth month; Fig. 1D). After one and seven months of incubation, only leachates from S-A12 (EC: 1.9 mS/cm) and S-A30 (EC: 3.1 mS/cm), respectively, presented EC significantly different from control (2.9 and 3.8 mS/cm). However by the end of the experiment (13 months), the EC in leachates from all SW-Technosols (3.6−4.7 mS/cm) were lower than control (5.2 mS/cm).

In general, no clear distinction was observed for doses application of 12 and 30 g/kg and type of amendment mixtures in SW-Technosols. However, the Technosol S-A60 seems to have more effective results in the pH increase and reduction of EC in leachates.

Anions concentrations in the leachates

Anions concentrations in the leachates from SW and the respective Technosols are given in Figure 2. The concentrations of anions in the simulated leachates were in the same range than those reported for acid mine drainage from the IPB (Abreu et al., 2008, 2010; Sánchez-España et al., 2005, 2008). Moreover, sulfates concentration in leachates from the first sampling (after one month of



SW: sulfide-rich wastes; S-A12, S-A30, S-A60: Technosol containing SW and AgW+AW+RW at 12, 30 and 60 g/kg; S-B12, S-B30: Technosol containing SW and AgW+CW+RW at 12 and 30 g/kg; S-C12, S-C30: Technosol containing SW and AgW+AW+CW+RW at 12 and 30 g/kg; AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops.

Fig. 1 Variation with time of pH and electrical conductivity in simulated leachates from Technosols and *gossan* wastes (Mean ± SD; $n = 3$), after 15 min (A and C) and 24 h (B and D) of agitation. Values from same sampling period followed by a different letter are significantly different ($p < 0.05$).

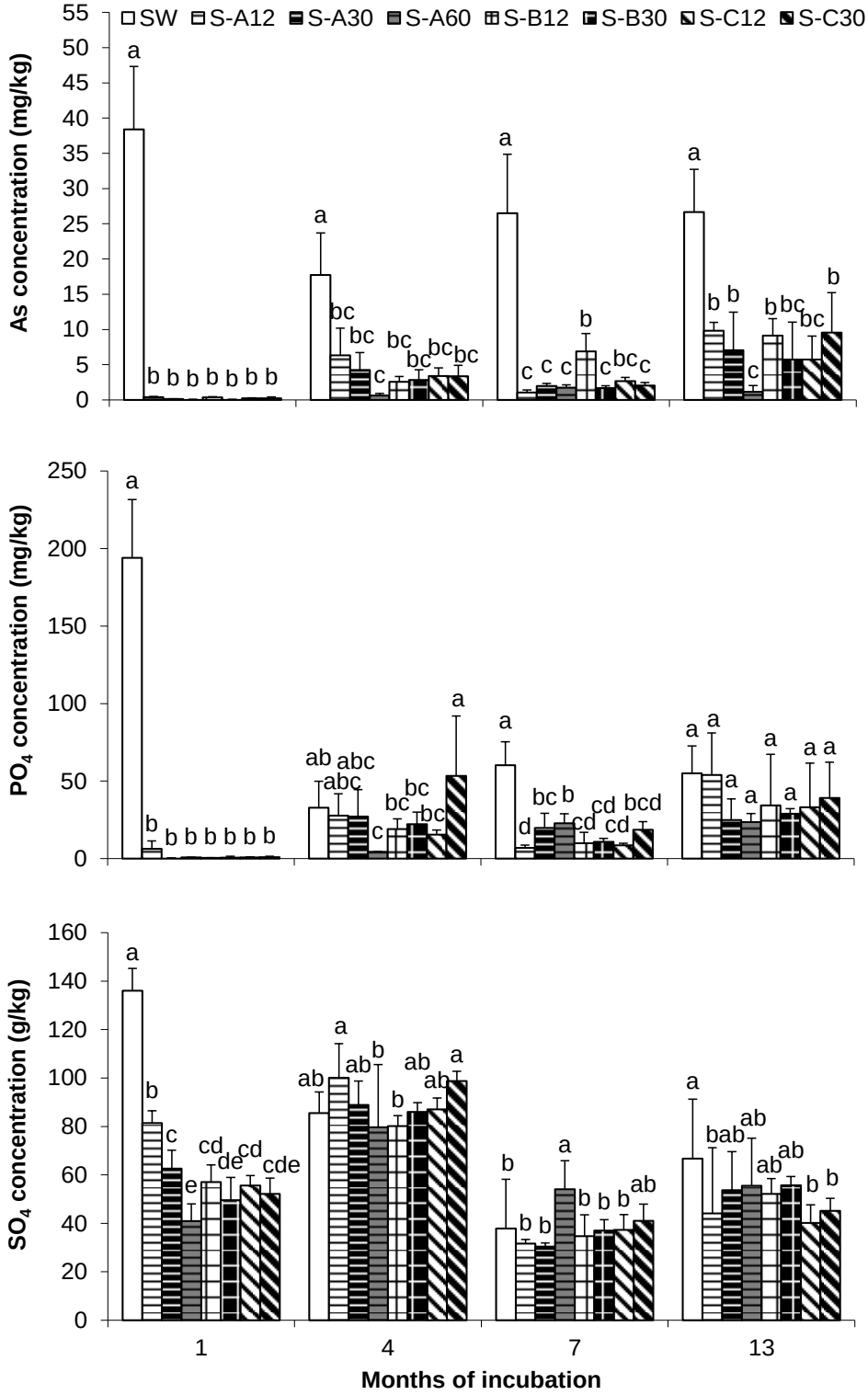
Incubation, 45.4 g S/kg) was still higher than those reported for the water/acid soluble and exchangeable fraction of the brittle pyrite wastes from São Domingos mine (34.4 g S/kg; Pérez-López et al. 2008). In the control, the concentrations of As (17.7−38.4 mg As/kg ≈ 1.5−4.8 mg/L) and sulfate (37.9−136.1 g/kg ≈ 3.2−11.2 g/L) were larger than in the majority of the simulated leaching (1:20 *m*:*V*) from mineralized rock samples collected in Furtei gold mine (0.001−0.27 mg As/L and 0.3−1.97 g SO_4/L; Da Pelo et al., 2009). However, the same authors also reported 10 mg As/L in the leachates of some samples. These variations can be related to the heterogeneous composition of the sulfide-rich wastes.

In general, significant differences were observed in the anions concentrations of leachates from SW-Technosols and SW. During the experiment, As concentrations in the leachates from all SW-Technosols, independently of the type and dose of amendments, decreased more than 63 % (mg/kg − Control: 17.7−38.4; SW-Technosols: 0.05−9.8). However, for phosphates and sulfates, this behaviour was only observed in some sampling periods. After one month of incubation, the SW-Technosols leachates also presented lower concentrations of phosphates and sulfates (0.2−6.3 mg PO_4/kg and 40.9−81.4 g SO_4/kg) than the control (194.1 mg PO_4/kg and 136.1 g SO_4/kg). Only in the leachates sampled in the seventh month of incubation was observed the same behaviour for phosphates (mg/kg − Control: 60.3; SW-Technosols: 6.9−22.7). Nevertheless the leachates collected after four months of incubation from SW-Technosols with high dose application of amendment mixture (S-A60) contained less phosphates than those collected from the control and the other SW-Technosols (Fig. 2).

After one month of incubation, the significant increase of the pH values in the leachates from SW-Technosols seems to influence negatively the concentrations of phosphates and sulfates ($r_{phosphate}$ = –0.72 and $r_{sulfate}$ = –0.83). The decrease of the anions concentrations can be explained by the crystallisation of metal arsenates (*e.g.* segnitite [$Pb(Fe^{3+})_3H(AsO_4)_2(OH)_6$)] and arsenbrackebuschite [$Pb_2(Fe^{2+},Zn)(AsO_4)(HAsO_4)(OH)$]),

phosphates (*e.g.* berlinite ($AlPO_4$) and corkite [$Pb(Fe^{3+})_3(PO_4)(SO_4)(OH)_6$]), and sulfates (*e.g.* alunogen [$Al_2(SO_4)_3$•$17H_2O$], linarite [$Cu^{2+}Pb(SO_4)(OH)_2$] and melanterite [$Fe^{2+}SO_4$•$7H_2O$]) both in the surface and/or in the core materials contained in the pots (Santos et al. 2014a). In general, the SW-Technosols, independently of the type and dose of amendments, were efficient in the decrease of the anions spread to the surrounding environment.

Significant variations in the anions concentrations occurred in all treatments during the experiment, however this fact was not explained only by pH values variation from leachates, but by the variation of all the elements concentrations, which can be saturated in relation to several solid phases. In general, significant increase of anions in leachates from SW-Technosols occurred between the first and fourth month of incubation as a result of the pH decrease.



SW: sulfide-rich wastes; S-A12, S-A30, S-A60: Technosol containing SW and AgW+AW+RW at 12, 30 and 60 g/kg; S-B12, S-B30: Technosol containing SW and AgW+CW+RW at 12 and 30 g/kg; S-C12, S-C30: Technosol containing SW and AgW+AW+CW+RW at 12 and 30 g/kg; AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops.

Fig. 2 Variation with time of anion concentrations in simulated leachates from Technosols and *gossan* wastes (Mean ± SD; $n = 3$). Values from same sampling period followed by a different letter are significantly different ($p < 0.05$).

Cations concentrations in the leachates

As for anions, the sulfide-rich wastes released great amounts of several cations (Fig. 3). Taking into account both seasonal and mine variability, simulated leachates from the SW (control) had elements concentrations in the same range than acid mine drainage from the IPB (Abreu et al., 2008, 2010; Sánchez-España et al., 2005, 2008). In general, cations concentrations (Cu, Fe, Mn, Pb and Zn) obtained in this study were smaller than those corresponding to water/acid soluble and exchangeable fraction in brittle pyrite wastes (379 mg Cu/kg, 19 mg Mn/kg and 494 mg Zn/kg; Pérez-López et al. 2008), although some exceptions were observed for specific elements and sampling periods (*e.g.* Cu, Mn and Zn after one month of incubation and Mn in the thirteenth month).

Concentrations of Ca (4.4−6.4 g/kg ≈ 386.4−529.5 mg/L) in the SW leachates were larger than those in leachates from Furtei mine (45−162 mg Ca/L; Da Pelo et al., 2009). However, in simulated leachates (1:20 *m*:*V*) from mineralized rocks collected in Furtei mine dumps (Da Pelo et al., 2009), the concentrations of Al, Cu, Fe and Na were in the same range than those in the leachates obtained from the SW. The same authors reported smaller concentrations of Zn (0.17−1.8 mg/L; studied mine wastes (SW): 11.6−63.0 mg/L ≈ 128.9−767.2 mg/kg) than in the control (SW), while concentrations of Pb and Mg were more than 200-fold higher than in the control (SW) (SW: 0.2−1.9 mg/L ≈ 2.5−21.0 mg/kg; 5.1−24.9 mg/L ≈ 56.8−301.3 mg/kg, respectively).

Significant differences were observed among the concentrations of some cations in leachates from the SW-Technosols and SW (Fig. 3). After one month of incubation, the amendment mixtures included in the SW-Technosols contributed to the improvement of the leachates quality. Thus, in these leachates, the concentrations of Al, Cu, Fe, Na and Zn decreased between 50 and 99 %, depending on the element, when compared to those from the control (SW: 2.1 g Al/kg; 589.7 mg Cu/kg; 7.9 g Fe/kg; 5.3 mg Na/kg; 767.2 mg Zn/kg). Although the immobilization of these cations can be related to their chelation, complexation and/or increase of exchangeable positions by organic matter addition (Adriano et al., 2004; Kumpiene et al., 2008), the increase of pH in the leachates (Fig. 1B), due to the acid neutralization by limestone rock wastes, seems to affect negatively the cations concentrations (r_{Al} = –0.88; r_{Cu} = –0.82; r_{Fe} = –0.73; r_{Zn} = –0.83). In fact, leaching of Cu and Zn is strongly pH dependent (Kumpiene et al., 2008).

The addition of phosphate to the waste materials, through the nutrition solution included in the rockwool and other organic wastes, can also contribute to the formation of metal-phosphates and consequently to the immobilization of the metals (Adriano et al. 2004; Hodson et al. 2001). In fact, solid phases as corkite [$Pb(Fe^{3+})_3(PO_4)(SO_4)(OH)_6$] and berlinite ($AlPO_4$) crystallised in the core of the same sulfide materials amended with the mixtures AW + AuW + CW + RW applied at 12 and 30 g/kg (corresponding to Technosols S-C12 and S-C30; Table 1) (Santos et al., 2014a).

The Na concentrations (< 0.4−2.8 mg/kg) in the SW-Technosols leachates were always very low when compared to the SW during all the experiment (50−99 % decrease of Na).

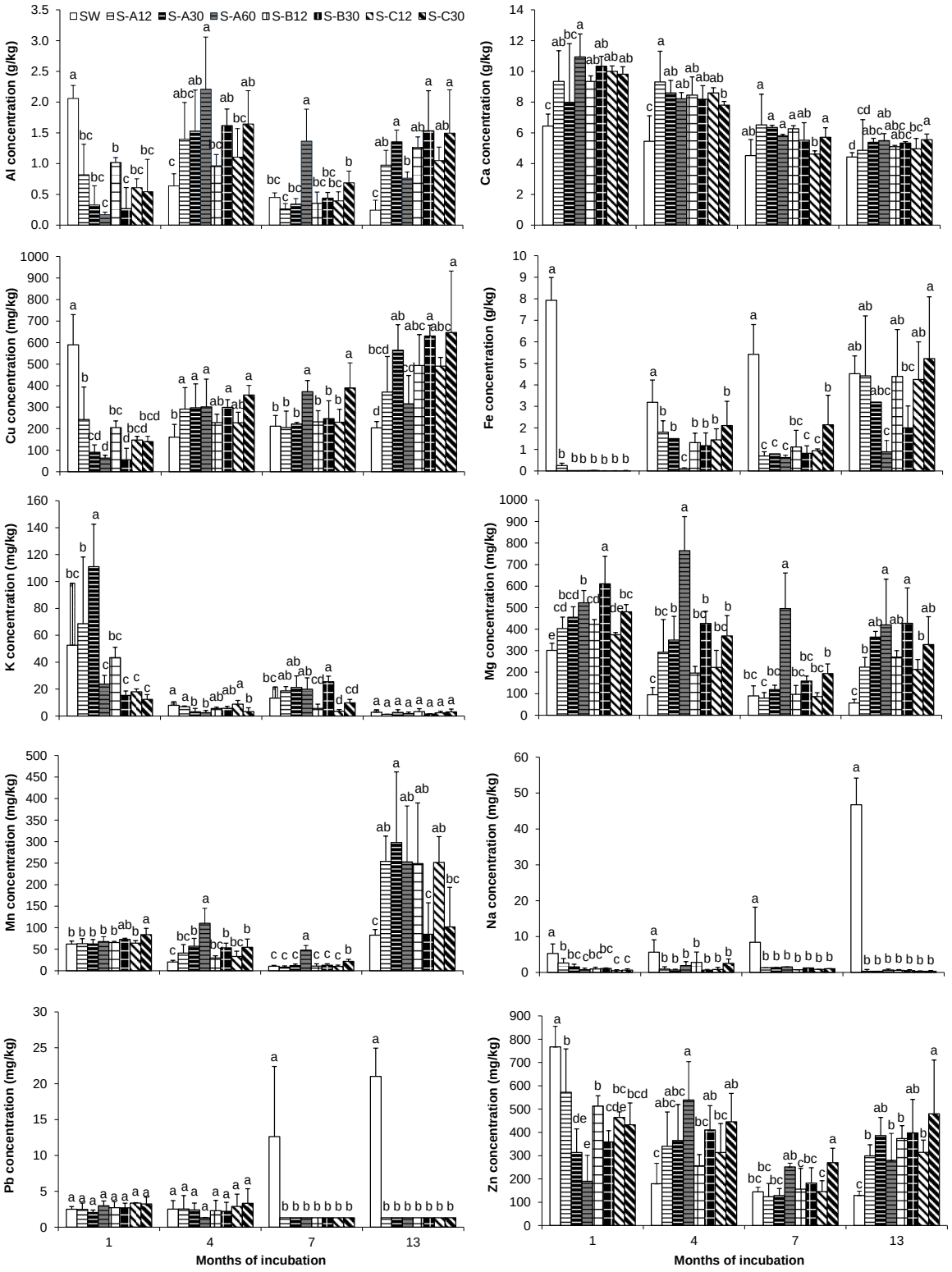
Copper and Zn concentrations in some SW-Technosols leachates were higher (205.5−646.9 mg Cu/kg; 123.5−538.7 mg Zn/kg, depending on sampling period) than in the SW (160.7−211.5 mg Cu/kg; 128.9−179.6 mg Zn/kg). This is probably due to the presence of low molecular weight organic

acids liberated during the amendments organic matter decomposition, accelerated by the sulfuric acid generated from the oxidation of pyrite. This organo-metallic complexes are water soluble and mobile (Kabata-Pendias and Pendias, 2001) and can explain the increase of Cu and Zn in the leachates of the SW-Technosols with time. The concentration of Fe in the SW-Technosols, after one month of incubation, is dramatically lower than in the SW leachates, what can be explained by the pH increase as a consequence of the limestone rock waste reaction with the acid generated by pyrite oxidation. The effect of the increase of the pH was not so drastic in decreasing the concentration of Cu and Zn in the leachates from the SW-Technosols collected one month after the incubation (Fig. 3). The Fe concentrations were progressively increasing during the time span of the experiment due to the combined effects of the pH decrease (as a consequence of limestone rock entire dissolution) and continuous Fe release from the pyrite oxidation.

After fourth and thirteen months of incubation, the concentrations of Al in SW-Technosols leachates were higher than in the SW. However, no clear tendency was observed related to the amendment mixture and application dose. The use of rockwool, with high amount of Al (Tables 1 and 2), in the amendment mixtures, and the low pH of the leachates collected after the first month of incubation (Fig. 1) can explain the increase of Al concentrations in the SW-Technosols leachates in relation to those collected from SW. In spite of the possible dissolution of the rockwool used in the amendments with release of Al to the acid solutions, the lower concentrations of the Al in the leachates of the SW-Technosols collected after one month of incubation, when compared to those of the leachates from SW, can be explained by the variations of the pH of the leachates. The solution with higher pH has lower concentrations of Al (Figs. 1 and 3).

Until the fourth month of incubation all the leachates from SW and SW-Technosols had similar concentrations of Pb (Fig. 3). After the seven month of incubation a positive contribution of the amendments included in the SW-Technosols was observed decrease of more than 87 % of the Pb concentration in the SW-Technosols leachates. The application of organic matter can promote the formation of organometallic complexes with Pb and the increase of the cationic exchange capacity of the materials (Adriano et al., 2004). The existence of phosphates (from organic wastes and nutrient solution included in the rockwool) can also justify the Pb concentration diminution in the leachates (Hodson et al. 2001). In fact, Santos et al. (2014a) reported not only berlinite ($AlPO_4$) but also other solid phases containing Pb, mainly belonging to the alunite–jarosite-group (*e.g*. plumbojarosite [$Pb(Fe^{3+})_6(SO_4)_4(OH)_{12}$], and segnitite [$Pb(Fe^{3+})3H(AsO_4)_2(OH)_6$]). Significant decrease of Pb concentrations in the leaching solutions was also obtained by Ioannidis and Zouboulis (2005) when synthetic and natural apatite was applied for the stabilization of simulated Pb-contaminated soils.

The concentrations of Ca and Mg in the leachates from SW-Technosols (4.9−10.9 g/kg and 221.9−764.5 mg/kg, respectively) had larger values than control (4.4−6.4 g Ca/kg and 56.8−301.3 mg Mg/kg) in all sampling periods (Fig. 3), as the result of the high concentrations of these elements in all the used amendments (Table 2). The rate of reductive dissolution of Mn oxides by organic matter is increased by lowering of pH (Sparks, 1995), which associated to the higher concentrations of Mn in the amendments can explain the increase of Mn in the leachates from SW-Technosols after thirteen months of incubation (Table 2 and Fig. 3).



SW: sulfide-rich wastes; S-A12, S-A30, S-A60: Technosol containing SW and AgW+AW+RW at 12, 30 and 60 g/kg; S-B12, S-B30: Technosol containing SW and AgW+CW+RW at 12 and 30 g/kg; S-C12, S-C30: Technosol containing SW and AgW+AW+CW+RW at 12 and 30 g/kg; AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops.

Fig. 3 Variation with time of cation concentrations in simulated leachates from Technosols and *gossan* wastes (Mean ± SD; $n = 3$). Values from same sampling period followed by a different letter are significantly different ($p < 0.05$)

Technosols composed of gossan wastes and amendment mixtures

Leachates electrical conductivity and pH

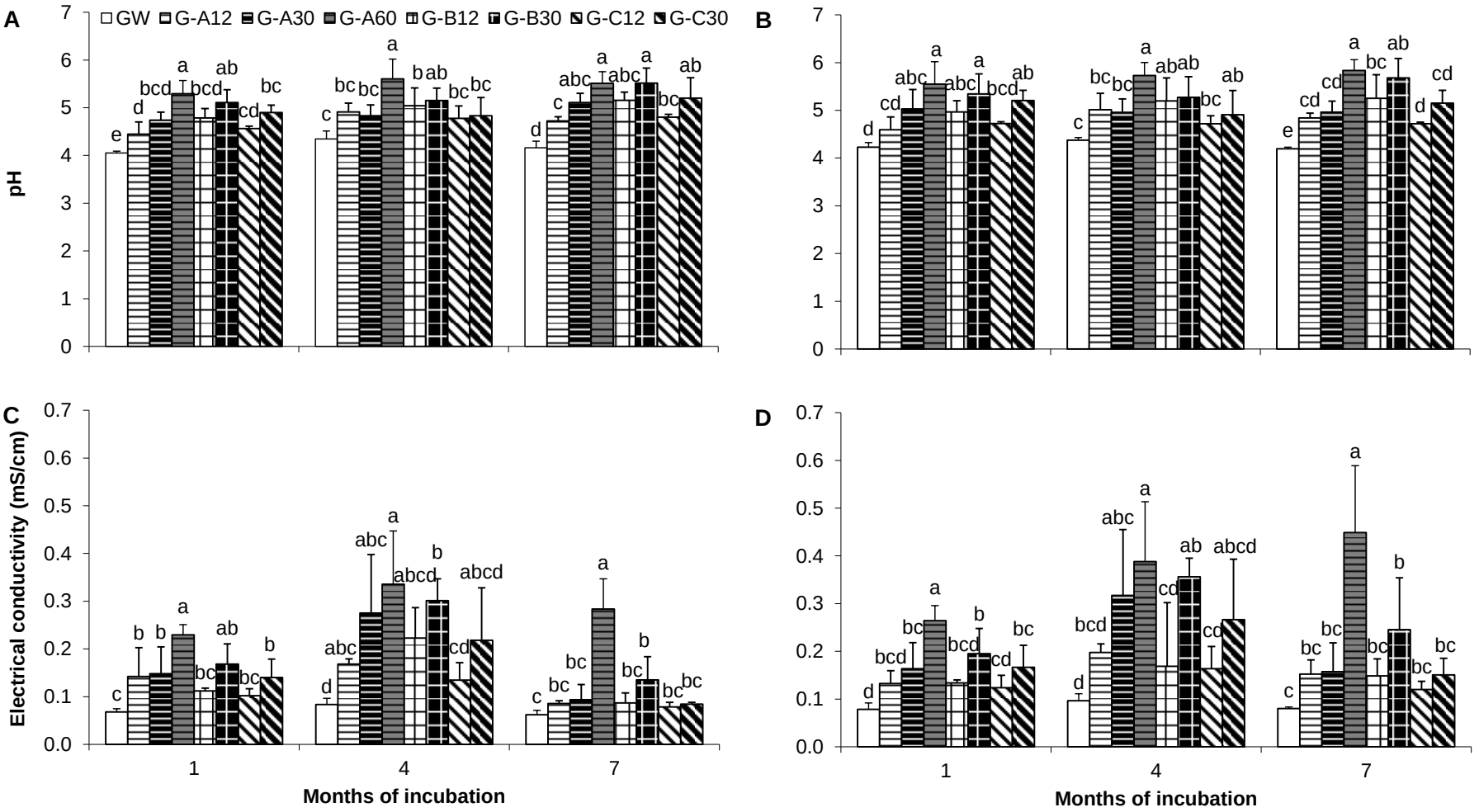
In the leachates obtained from the GW and respective Technosols, and for the same treatment, similar pH and EC values were observed after 15 min and 24 h of shaking (Fig. 4). The values of pH and EC obtained from GW leachates were different from those reported by Santos et al. (2013) to soils developed on gossan materials collected in the same mine area.

The leachates pH and EC, for all the sampling periods and amendments application increased during the experiments' time (Control − pH: 4.2−4.4 and EC: 0.08−0.10 mS/cm, GW-Technosols − pH: 4.6−5.8 and EC: 0.12−0.45 mS/cm; Figs. 4B and 4D). Similar behaviour was reported by Alvarenga et al. (2009), by the application of different organic residues (municipal solid waste compost, garden waste compost and sewage sludge), at 25, 50 and 100 Mg/ha (doses close to those applied in this study – 30, 75 and 150 Mg/ha), to metal-contaminated soils from the Aljustrel mining area. However, Santos et al. (2013) found that for soils collected in the São Domingos mine and developed on *gossan* wastes amended with hydrophilic polyacrylate polymers occurred an increase of the pH of the leachates (Control: 6.1; Amended treatments: 6.9−7.1) while the EC in all the cases remained almost constant (≈0.06 mS/cm).

After one month of incubation, significant differences were already obtained in pH and EC of the leachates from the GW-Technosols containing the 30 and 60 g/kg doses of amendments (pH: 5.0−5.5, EC: 0.16−0.26 mS/cm), when compared with the leachates from GW (pH: 4.2, EC: 0.08 mS/cm) (Figs. 4B and 4D). However, after seven months, this significant improvement of pH was obtained in all leachates from the GW-Technosols independently of the application dose and the amendment mixture (pH: 4.8−5.8, Fig. 4B). In the same period, only leachates from Technosols G-A60 (EC: 0.45 mS/cm) and G-B30 (EC: 0.25 mS/cm), which received a high dose of amendment and with specific amendment mixture (residue from the liquor distillation of *A. unedo* fruit and amendment application at 60 g/kg and residue from liquor distillation of *C. siliqua* fruit and amendment application at 30 g/kg, respectively), had higher EC than the control (0.08 mS/cm). Although EC had increased slightly in leachates from Technosols when compared with control (GW), these values were still small (< 0.45 mS/cm). No variation in the values of pH and EC in the leachates from each treatment were observed along the time.

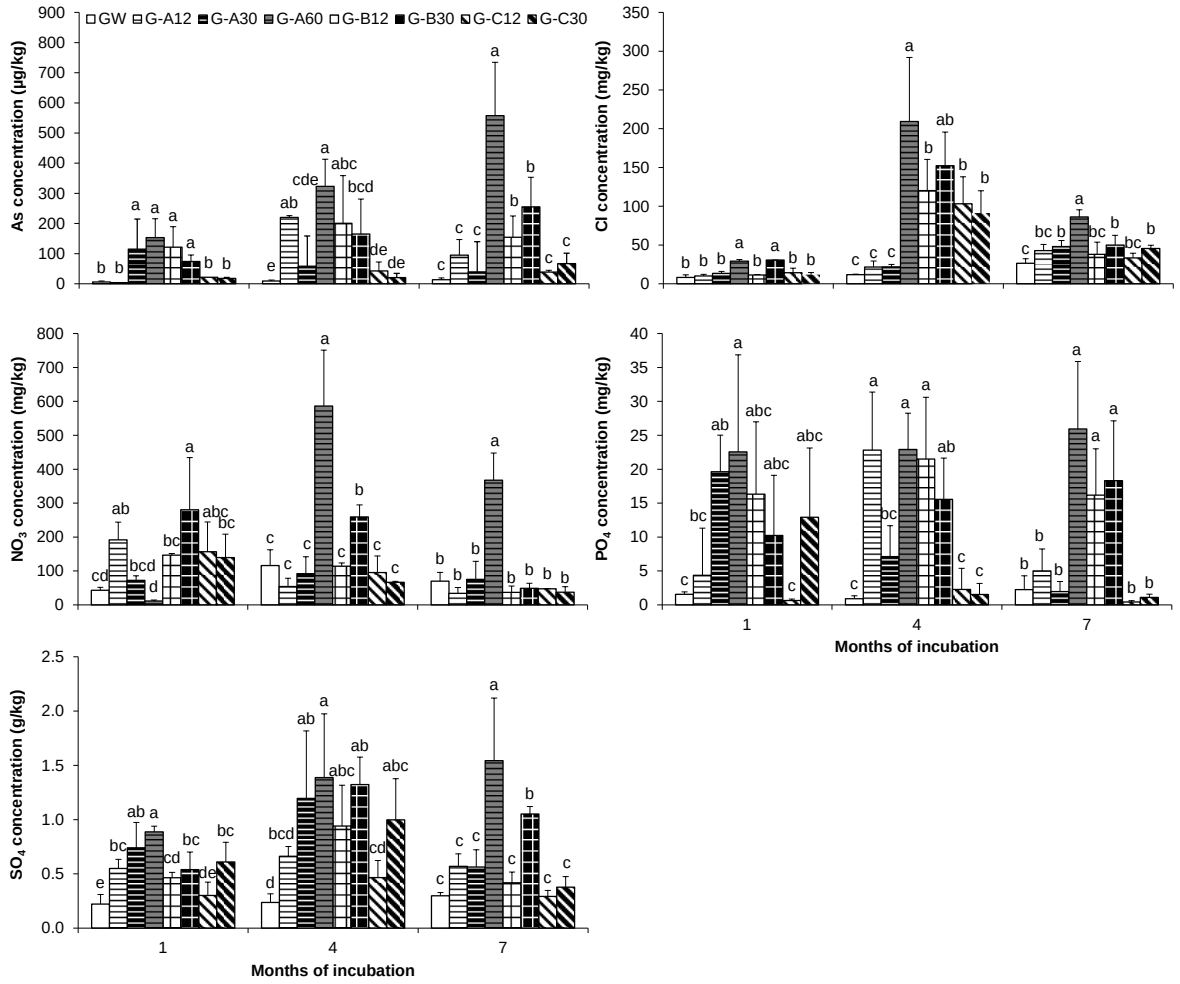
Anions concentrations in the leachates

Anions concentrations in the leachates from GW and respective GW-Technosols are shown in Figure 5. In all sampling periods, leachates from some GW-Technosols had higher anions concentrations than GW, but no clear tendency was observed for the amendment mixture and application dose of 12 and 30 g/kg. Nevertheless, GW-Technosol containing the amendment mixture at 60 g/kg (G-A60), usually released, by leaching, the highest amount of anions. The increase of chlorides, nitrates and phosphates in leachates from GW-Technosols is mainly related to the nutrient solution used in the strawberry cultivation, which was incorporated in rockwool and agriculture wastes (Tables 1 and 2).



GW: *gossan* wastes; G-A12, G-A30, G-A60: Technosol containing GW and AgW+AW+RW at 12, 30 and 60 g/kg; G-B12, G-B30: Technosol containing GW and AgW+CW+RW at 12 and 30 g/kg; G-C12, G-C30: Technosol containing GW and AgW+AW+CW+RW at 12 and 30 g/kg; AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops.

Fig. 4 Variation with time of pH and electrical conductivity in simulated leachates from Technosols and *gossan* wastes (Mean ± SD; $n = 4$), after 15 min (A and C) and 24 h (B and D) of agitation. Values from same sampling period followed by a different letter are significantly different ($p < 0.05$)



GW: *gossan* wastes; G-A12, G-A30, G-A60: Technosol containing GW and AgW+AW+RW at 12, 30 and 60 g/kg; G-B12, G-B30: Technosol containing GW and AgW+CW+RW at 12 and 30 g/kg; G-C12, G-C30: Technosol containing GW and AgW+AW+CW+RW at 12 and 30 g/kg; AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops.

Fig. 5 Variation with time of anion concentrations in simulated leachates from Technosols and *gossan* wastes (Mean ± SD; $n = 4$). Values from same sampling period followed by a different letter are significantly different ($p < 0.05$).

Although the concentrations of anions in the leachates are different, compared to the present study, the increase of As and phosphate in leachates from soils developed on *gossan* materials amended with polyacrylates was also observed (Santos et al., 2013). However, according to the same study, the same increase did not occur for chlorides, nitrates and sulfates.

The higher As concentrations in leachates from GW-Technosols (21.4−557.8 µg/kg depending on treatment and sampling period) can be related to the increase of low-molecular-weight organic acids, derived from amendments application, which can release As from Fe–, Mn– and Al–oxides or hydroxides (Zang et al., 2005). In fact, soils developed on this mine waste in São Domingos mining area have 3–34 % and 0.01−0.4 % of the total As associated to Fe–oxides and Mn-oxides, respectively (Abreu et al., 2012; Santos et al., 2012). Another explanation to the increase of As concentration in the leachates can be given by the presence of solid phases with low solubility, at lower pH, that can naturally exist in the *gossan* materials, like carminite ($Fe_2Pb(AsO_4)_2(OH)_2$), segnitite

($Fe_3Pb(AsO_4)(HAsO_4)(OH)_6$) and kankite ($FeAsO_4•3.5H_2O$) (Santos et al., 2012). The solubility of these solid phases increases with the increasing of the pH of their environment, as a result of their non-congruent dissolution. The increase of As concentrations in those leachates can be related to the complex behaviour of the systems where new solid phases can be formed with different solubilities.

The time evolution of the leachates anions concentrations showed no significant variations in the concentrations of As (except for G-A12), phosphate (except for G-A12) and sulfate, independently of the treatment. In general, chloride concentrations reached the highest values at the fourth month of incubation. For nitrates, it is apparent a decrease in their concentrations, but no clear tendency was observed according to amendment dose and/or mixture.

Cations concentrations in the leachates

Cations concentrations in leachates from GW and respective Technosols are shown in Figure 6 (except Al, Cu and Pb). All leachates had concentrations of Pb (< 2.5 mg/kg), Al (in first and fourth month < 2.8 mg/kg) and Cu (seventh month < 0.5 mg/kg) below the detection limit of the apparatus.

Concentrations of the studied cations in leachates from GW were quite different from those reported by Santos et al. (2013). These variations will be related to the chemical composition of the waste materials and despite being both classified as *gossan* materials, their heterogeneity is well known.

Similar concentrations of Zn after one and seven months of incubation and Fe after one and four months of incubation were observed among treatments. However, Alvarenga et al. (2009) reported a decrease of Zn in simulated leaching assays carried out in contaminated soils from Aljustrel mine area where organic wastes were applied.

Amendments application, independently of application dose and type, decreased significantly the Cu concentrations (> 50 %) after one and four months, compared to control (≈0.7 mg/kg), to values lower than detection limit. Similar results were observed by Alvarenga et al. (2009). In fact, organic matter application can promote the formation of stable complexes with Cu and increase cationic exchange capacity (Adriano et al., 2004; Kumpiene et al., 2008).

Leachates from the GW-Technosols had higher concentrations of Ca, K, Mg, Mn and Na (more than 20 %) than the control (mg/kg – Ca: 21.7−62.7, K: 41.0−64.7, Mg: 6.0−10.8, Mn: <0.5−0.6, Na: 5.3−25.0), especially after one and four month of incubation. All the amendments mixtures and application doses originated the increase of Ca concentration in the leachates during all the experiment. For the other cations, a different behaviour occurs and no clear tendency was observed whatever the amendment mixture and/or application dose. Similarly to some anions behaviour, the high concentrations of these nutrients in leachates from the GW-Technosols were mainly related to the nutrient solution incorporated in the rockwool (Tables 1 and 2). The increase of Fe concentration in the leachates was also observed in soils developed on *gossan* materials amended with diapers (Santos et al., 2013). These authors also observed that the application of different types of polyacrylate did not alter the concentrations of Ca, K, Mg, Mn and Na in the leachates.

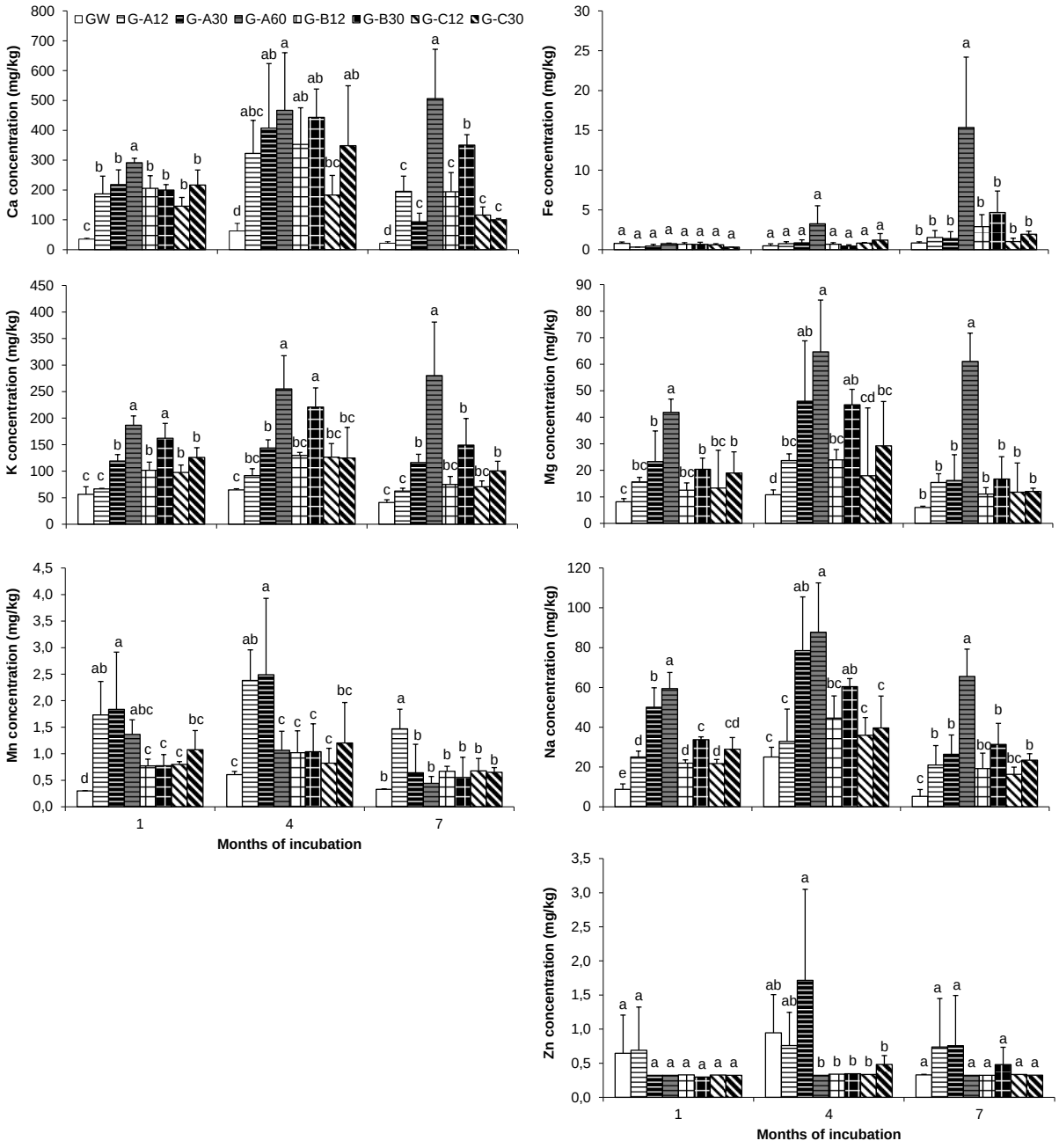


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Fig. 6 Variation with time of cation concentrations in simulated leachates from Technosols and *gossan* wastes (Mean ± SD; $n = 4$). Values from same sampling period followed by a different letter are significantly different ($p < 0.05$).

Environmental impact

Simulated leachates reflected the sulfide reactivity of the mine wastes. Although various factors (*e.g*. climate conditions, design of the fill site and presence of vegetation) can control the leaching of

the elements from wastes/soils under field conditions (Chezom et al., 2013), it is essential the evaluation of the mobilization of hazardous elements by the leachates and their potential environmental impact. Considering the concentrations of the potentially hazardous elements obtained in the simulated leachates and the total mass of GW and SW estimated in São Domingos mine (Tg − SW: 0.20−0.27 and GW: 4.1−4.34; Álvarez-Valero et al., 2008; Pérez-López et al., 2008), the total amounts of the elements released by the mine wastes are presented in Table 3.

Sulfide-rich wastes can leach to the adjacent areas significant amounts of potentially hazardous elements. In general, the amounts of these elements were significantly lower than the values obtained by Pérez-Lopez et al. (2008), which considered the available fraction (soluble and exchangeable fraction) of the same elements in the wastes. Soluble fractions of Al, As, Fe and Pb represented less than 7 % of the total concentrations of these elements, but other chemical elements, that can become hazardous, are mainly available (percentage of the available fraction compared to their total concentrations − S: 19−70; Cu: 8−28; Zn: 11−68).

In spite of the low values of the soluble fractions in the GW and respective Technosols (< 2.6 % of total concentrations depending on the element, treatment and sampling period) and of the elements concentrations in the leachates (with or without amendments application), in each rainfall also can be leached significant amounts of hazardous elements for the adjacent areas due to the great total mass of this type of mine wastes that exist in São Domingos mine.

Although the total mass of SW is smaller than the mass of GW in the mine area, the high concentrations of potentially hazardous elements leached from SW represent a greatest environmental impact in each rainfall. The circulation of water and leachates through SW, which generate acids by sulfides oxidation, amplified the environmental impact when compared to other mine wastes.

In general, the conception of GW-Technosols increases the amounts of some elements in the leachates. However, in the GW-Technosols, especially with application of the highest dose of the amendments, the increase of organic matter and nutrient contents stimulate the development/colonization of vegetation (Santos et al., 2014b, 2015) in these soils. Consequently, this can reduce the chemical elements leaching and the water flow to the deeper zones of the tailings reducing the wastes-water interaction. Nevertheless, Santos et al. (2015) referred that no vegetation was able to grow in the SW-Technosols owing to the very low pH, as a result of continuous acid drainage generation and low buffer capacity of the amendments.

Comparison of methodologies to obtain leachates

Comparing the elements concentrations in the leachates obtained by the DIN method with those in the leachates obtained by distilled water percolation (Santos et al., 2014a, 2015), it is evident the high availability of the chemical elements in the *gossan* (GW) and sulfide wastes (SW). Although the elements' concentrations in the leachates from SW are in the same range than the values reported for acid mine drainage from IPB (Abreu et al., 2008, 2010; Sánchez-España et al., 2005, 2008), significant differences between the elements concentrations in the leachates obtained by the two

above referred methods were observed for the majority of the elements. The same variation was obtained for the concentrations of the elements in the leachates from *gossan* wastes obtained with the two referred methodologies.

Even being the DIN extraction a forced extraction method (Gomes and Pinto, 2006), the concentrations of the elements in the simulated leachates were significantly smaller than in leachates obtained by water percolation (Santos et al., 2014a, 2015), independently of the mine wastes and consequently their reactivity. Similar results were obtained with municipal solid wastes by Kocasoy and Murat (2009) but not with steelmaking slags (Gomes and Pinto, 2006).

Several factors can be associated to this variation between leaching methodologies, as for instance the sample size, the particle size distribution, the solid:liquid ratio, and the duration of the leaching test (Chezom et al., 2013; Fällman and Aurell, 1996; Gomes and Pinto, 2006). The size of the waste particles used in each method (< 2 mm in the DIN method and the total fraction of SW or < 1 cm for GW in the water percolation method), and the mechanic agitation can be considered the main origin of the differences between the values of the elements concentrations in the leachates. The influence of particle size in batch test was demonstrated by Fällman and Aurell (1996) for lead refractory slags.

The heterogeneity of the solid phases composing the mine wastes and consequently the surface are exposed to the water flow in the pots is larger, while the ratio solid:liquid is much smaller in the percolation method (1:0.08 *m*:*V*) than in the DIN extraction (1:10 *m*:*V*). In general, the water percolates through preferential channels, due to the large grain size of mine wastes.

It should be noticed that during the experiment can occur several chemical processes, like complexation and/or adsorption/desorption (Gomes and Pinto, 2006) and even precipitation of secondary minerals (Fällman and Aurell, 1996; Santos et al., 2014a) what can influence the concentration of the elements in the simulated leachates. Nevertheless in a cycle of leaching-evaporation, different solid phases can be formed with stabilities determined by the environment, which can reduce or increase the elements concentrations according to the environment changes. In fact, in the percolation experiments with SW was identified a significant time evolution of the efflorescent salts and in solid phases in the pots' core materials (Santos et al., 2014a).The variation in the primary and secondary minerals with different solubilities will change the concentrations of the elements in the leachates (Fällman and Aurell, 1996; Santos et al., 2014a).

Table 3 Mass (Mg) of elements leached from the sulfide-rich and *gossan* wastes and respective Technosols based on the concentrations of elements in simulated leachates and total mass of tailings in São Domingos mining area

	Al	As	Cu	Fe	Pb	S	Zn
				Assay 1 (Gossan wastes)			
GW	<11.7	0.02 − 0.1	<2.2 − 3.4	2.1 − 3.7	<10.9	0.9 − 1.3	<2.1 − 4.1
G-A12	<11.7	0.01 − 1.0	<2.2	<2.1 − 6.6	<10.9	2.3 − 2.9	2.8 − 3.3
G-A30	<11.7	0.2 − 0.5	<2.2	<2.1 − 6.1	<10.9	3.0 − 5.2	<2.1 − 7.4
G-A60	<11.7 − 20.9	0.6 − 2.4	<2.2	3.1 − 66.6	<10.9	3.6 − 6.0	<2.1
G-B12	<11.7	0.5 − 0.9	<2.2	2.8 − 12.5	<10.9	1.9 − 4.1	<2.1
G-B30	<11.7	0.3 − 1.1	<2.2	<2.1 − 20.3	<10.9	2.2 − 5.7	<2.1
G-C12	<11.7	0.1 − 0.2	<2.2	2.5 − 4.5	<10.9	1.2 − 2.0	<2.1
G-C30	<11.7	0.1 − 0.3	<2.2	<2.1 − 8.4	<10.9	2.5 − 4.3	<2.1
				Assay 2 (Sulfide-rich wastes)			
SW	48.6 − 556	3.5 − 10.4	32.1 − 159	637 − 2140	0.5 − 5.7	2531 − 12265	29.0 − 207.1
S-A12	52.6 − 377	0.1 − 2.6	41.1 − 100	49.9 − 1193	<0.5 − 0.7	2108 − 7335	24.7 − 154
S-A30	68.1 − 412	0.02 − 1.9	18.0 − 153	2.9 − 863	<0.5 − 0.8	2027 − 8008	25.7 − 104
S-A60	33.8 − 597	0.01 − 0.5	12.6 − 100	3.0 − 240	<0.5 − 0.7	3608 − 7177	38.0 − 145
S-B12	70.7 − 340	0.1 − 2.5	41.0 − 133	4.3 − 1185	<0.5 − 0.7	2318 − 7218	31.2 − 139
S-B30	53.7 − 436	0.01 − 1.5	11.2 − 170	1.4 − 540	<0.5 − 0.7	2469 − 7746	36.5 − 111
S-C12	78.7 − 299	0.05 − 1.5	29.4 − 133	2.0 − 1147	<0.5 − 0.9	2485 − 7840	29.1 − 125
S-C30	108 − 444	0.05 − 2.6	28.0 − 175	2.6 − 1410	<0.5 − 0.9	2741 − 8897	53.9 − 129

GW: *gossan* wastes; G-A12, G-A30, G-A60: Technosol containing GW and AgW+AW+RW at 12, 30 and 60 g/kg; G-B12, G-B30: Technosol containing GW and AgW+CW+RW at 12 and 30 g/kg; G-C12, G-C30: Technosol containing GW and AgW+AW+CW+RW at 12 and 30 g/kg; SW: sulfide-rich wastes; S-A12, S-A30, S-A60: Technosol containing SW and AgW+AW+RW at 12, 30 and 60 g/kg; S-B12, S-B30: Technosol containing SW and AgW+CW+RW at 12 and 30 g/kg; S-C12, S-C30: Technosol containing SW and AgW+AW+CW+RW at 12 and 30 g/kg; AgW: agriculture wastes; AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops

CONCLUSIONS

Based on elements concentrations in simulated leachates and total mass of *gossan* (GW) and sulfide-rich wastes (SW) in São Domingos mine, both mine wastes generate great environmental impact. The continuous oxidation of the sulfide minerals led to the formation of very acid solutions, which can release and afterwards leach high amounts of anions and cations. Although the GW had leached lower amounts of elements, compared to the SW, the extensive mass of the GW contribute to the release of considerable amounts of potentially hazardous elements. Being the annual precipitation in São Domingos relatively small (559 mm), the tailings can still release continuously in each rainfall event great amounts of potentially hazardous elements to the adjacent areas increasing the elements' concentrations in soils and waters.

The amendments application improved the GW, decreasing their environmental impact, but the beneficial effect of the amendments was not clear for the SW. However a clear influence of the dose and type of the amendments was not observed during the entire experiment. Although GW-Technosols had leached more As when compared to the control, its concentrations were small. Moreover, the increase of pH and nutrients in leachates from these Technosols is an advantage in the rehabilitation process. In SW-Technosols, the amendments mixtures did not increase the pH of the leachates since the fourth month of incubation, owing to the absence of limestone rock waste, because the carbonates were dissolved in the reaction with the sulfuric acid generated by the pyrite oxidation. Other organic and inorganic wastes and, possibly, their rate of application should be studied over a longer time span with the objective of increasing the pH of the leachates from SW, which seems to decrease the cations and anions concentrations in the leachates as was observed in the first month of incubation.

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REFERENCES

Abreu, M.M., Tavares, M.T., Batista, M.J., 2008. Potential use of *Erica andevalensis* and *Erica australis* in phytoremediation of sulphide mine environments: São Domingos, Portugal. Journal of Geochemical Exploration 96, 210-222.

Abreu, M.M., Batista, M.J., Magalhães, M.C.F., Matos, J.X., 2010a. Acid mine drainage in the Portuguese Iberian Pyrite Belt. In: Brock C.R. (Eds.), Mine drainage and related problems. Nova Science Publishers, Inc. New York, pp. 71-118.

Abreu, M.M., Santos, E.S., Ferreira, M., Magalhães, M.C.F., 2012a. Cistus salviifolius a promising species for mine wastes remediation. Journal of Geochemical Exploration 113, 86-93.

Adriano, D.C., Wenzel, W.W., Vangronsveld, J., Bolan, N.S., 2004. Role of assisted natural attenuation in environmental cleanup. Geoderma 122, 121-142.

Alvarenga, P., Palma, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2009. Organic residues as immobilizing agents in aided phytostabilization: (II) Effects on soil biochemical and ecotoxicological characteristics. Chemosphere 74, 1301-1308.

Álvarez-Valero, A.M., Pérez-López, R., Matos, J., Capitán, M.A., Nieto, J.M., Sáez, R., Delgado, J., Caraballo, M., 2008. Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian Peninsula): evidence from a chemical and mineralogical characterization. Environmental Geology 55(8), 1797-1809.

Chezom, D., Chimi, K., Choden, S., Wangmo, T., Gupta, S.K., 2013. Comparative study of different leaching procedures. International Journal of Engine Research 1(2), 5 pp.

Da Pelo, S., Musu, E., Cidu, R., Frau, F., Lattanzi, P., 2009. Release of toxic elements from rocks and mine wastes at the Furtei gold mine (Sardinia, Italy). Journal of Geochemical Exploration 100, 142-152.

DIN 38414-S4, 1984. Schlamm und Sedimente, Bestimmung der Eluierbarkeit mit Wasser. DIN Deutsches Institut für Normung, Berlin.

Fällman, A.M., Aurell, B., 1996. Leaching tests for environmental assessment of inorganic substances in wastes, Sweden. Science of Total Environmet 178, 71-84.

Forsberg, L.S., Gustafsson, J.P., Kleja, D.B., Ledin, S., 2008. Leaching of metals from oxidising sulphide mine tailings with and without sewage sludge application. Water, Air and Soil Pollution 194, 331-341.

Gomes, J.F.P., Pinto, C.G., 2006. Leaching of heavy metals from steelmaking slags. Revista de Metalurgia 42(6), 409-416.

González, V., Moral, G.F.D., Simón, M., 2012. Effectiveness of amendments on the spread and phytotoxicity of contaminants in metal–arsenic polluted soil. Journal of Hazardous Materials 205-206, 72-80.

Hodson, M.E., Valsami-Jones, E., Cotter-Howells, J.D., Dubbin, W.E., Kemp, A.J., Thornton, I., Warren, A., 2001. Effect of bone meal (calcium phosphate) amendments on metal release from contaminated soils − a leaching column study. Environmental Pollution 112, 233-243.

Ioannidis, T.A., Zouboulis, A.I., 2005. Hazardous industrial waste stabilization using inorganic phosphates: Investigation of possible mechanisms. Pure and Applied Chemistry 77(10), 1737-1752.

Kocasoy, G., Murat, Z.M., 2009. Comparison of the characteristics of the leachate and the eluates obtained by different methods. Journal of Environmental Science and Health 44(6), 605-614.

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments − a review. Waste Management 28, 215-225.

Macías, F., 2004. Recuperación de suelos degradados, reutilización de resíduos y secuestro de carbono. Una alternativa integral de mejora de la calidad ambiental. Recursos Rurais 1, 49-56.

Macías, F., Macías-García, F., Nieto, C., Verde, J.R., Pérez, C., Bao, M., Camps-Arbestain, M., 2011. Gestión de residuos y cambio climático. In: Mosquera, M.E.L., Osés, M.J.S., (Eds.), Gestión de residuos orgánicos de uso agrícola. Servizo de Publicacións e Intercambio Científico de la Universidade de Santiago de Compostela, Santiago de Compostela.

Matos, J.X., Martins, L.P., 2006. Reabilitação ambiental de áreas mineiras do sector português da Faixa Piritosa Ibérica: estado da arte e perspectivas futuras. Boletín Geológico y Minero 117, 289-304.

Perera, A.S., Al-Tabaa, A., Rid, J.M., Stegmemann, J.A., Shi, C., 2005. Testing and performance criteria for stabilized/solidified wastes forms. In: Spence, R.D., Shi, C. (Eds.) Stabilization and solidification of hazardous, radioactive, and mixed wastes. Boca Raton, CRC press.

Pérez-López, R., Álvarez-Valero, A.M., Nieto, J.M., Sáez, R., Matos, J.X., 2008. Use of sequential extraction procedure for assessing the environmental impact at regional scale of the São Domingos Mine (Iberian Pyrite Belt). Applied Geochemistry 23, 3452-3463.

Quental, L., Bourguignon, A., Sousa, A.J., Batista, M.J., Brito, M.G., Tavares, T., Abreu, M.M., Vairinho, M., Cottard, F., 2002. MINEO Southern Europe environment test site. Contamination impact mapping and modelling - Final Report. Assessing and monitoring the environmental impact of mining activities in Europe using advanced Earth Observation Techniques (MINEO) 5yth FP-IST-1999-10337.

Quental, L.E., Sousa, A.J., Marsh, S., Brito, G., Abreu, M.M., 2013. Identification of materials related to acid mine drainage using HYMAPTM at S. Domingos mine, SE Portugal. International Journal of Remote Sensing 34(6), 1928-1948.

Reis, A.P., Ferreira da Silva, E., Cardoso Fonseca, E., Patinha, C., Barrosinho, C., Matos, J., 2012. Environmental assessment of the Caveira abandoned mine (southern Portugal): Part 1: characterization of metal contaminated soil. Soil and Sediment Contamination: An International Journal 21, 2:227-254.

Sánchez-España, J., Pamo, E.L., Santofimia, E., Aduvire, O., Reyes, J.A, Barettino, D., 2005. Acid mine drainage in the Iberian Pyrite Belt (Odiel river watershed, Huelva, SW Spain): Geochemistry, mineralogy and environmental implications. Applied Geochemistry 20(7), 1320-1356.

Sánchez-España, J., Toril, G.E., Pamo, E.L., Amils, R., Ercilla, M.D., Pastor, E.S., San Martín-Úriz, P., 2008. Biogeochemistry of hyperacid and ultraconcentrated pyrite leachate in San Telmo mine (Iberian Pyrte Belt, Spain). Water, Air and Soil Pollution 194, 243-257.

Santos, E.S., Abreu, M.M., Nabais, C., Magalhães, M.C.F., 2012. Trace element distribution in soils developed on *gossan* mine wastes and *Cistus ladanifer* L. tolerance and bioaccumulation. Journal of Geochemical Exploration 123, 45-51.

Santos, E.S., Abreu, M.M., de Varennes, A., Macías, F., Leitão, S., Cerejeira, M.J., 2013. Evaluation of chemical parameters and ecotoxicity of a soil developed on *gossan* following application of polyacrylates and growth of *Spergularia purpurea*. Science of the Total Environment 461-462, 360-370.

Santos, E.S., Magalhães, M.C.F., Abreu, M.M., Macías, F., 2014a. Effects of organic/inorganic amendments on trace elements dispersion by leachates from sulfide-containing tailings of the São Domingos mine, Portugal. Time evaluation. Geoderma 226-227, 188-203.

Santos, E.S., Abreu, M.M., Macías, F., de Varennes, A., 2014b Improvement of chemical and biological properties of *gossan* mine wastes following application of amendments and growth of *Cistus ladanifer* L.. Journal of Geochemical Exploration 147, 173-181.

Santos, E.S., Abreu, M.M., Macías, F., de Varennes, A., 2015. Chemical quality of leachates and enzimatic activities in Technosols with *gossan* and sulfide wastes from the São Domingos mine. Journal of Soils and Sediments. DOI: 10.1007/s11368-015-1068-8

Sparks, D.L., 1995. Environmental soil chemistry. Academic Press, London.

USEPA – United States Environmental Protection Agency, 1979. Methods for chemical analysis of water and waste. Office of Research and Development, US Environmental Protection Agency, method 365.2, Cincinatti, Ohio.

Yao, F.X., Macías, F., Santesteban, A., Virgel, S., Blanco, F., Jiang, X., Camps Arbestain, M., 2009. Influence of the acid buffering capacity of different types of Technosols on the chemistry of their leachates. Chemosphere 74, 250-258.

Zhang, S., Li, W., Shan, X.Q., Lu, A., Zhou, P., 2005. Effects of low molecular weight organic anions on the release of arsenite and arsenate from a contaminated soil. Water, Air and Soil Pollution 167(1-4), 111-122.

7. EFFECTS OF ORGANIC/INORGANIC AMENDMENTS ON TRACE ELEMENTS DISPERSION BY LEACHATES FROM SULFIDE-CONTAINING TAILINGS OF THE SÃO DOMINGOS MINE, PORTUGAL. TIME EVALUATION

ABSTRACT

Greenhouse pot experiments were conducted over 13 months to evaluate the effect of two amendment mixtures doses (30 and 75 Mg/ha) on the geochemical dispersion of trace elements by leaching hazardous mine wastes from the São Domingos mine. Mineralogical evolution of these materials was also evaluated. Amendment mixtures containing solid wastes from agriculture (plant remains + strawberry substrate and rockwool used for the strawberry crop) and from distillation of *Ceratonia siliqua* L. and *Arbutus unedo* L. fruits were used to improve the chemical characteristics of leachates from sulfide mine wastes. Sulfide mine wastes had acidic characteristics, as well as high electrical conductivity and total elements concentrations (g/kg; Al: 54.8–61.2; Fe: 104.0–110.0; Pb: 9.0–13.8; S: 63.6–68.0; As: ≈1). These features contributed to the large capacity for leaching of hazardous elements during, at least, the first four months of incubation. In the seventh month of incubation, there was a significant decrease in the leachates concentration of the majority of hazardous elements. The addition of amendments minimised trace elements dispersion in leachates percolation during the first seven months (25 to 99 % reduction compared to control, depending on the element and sampling period). However, the leachates characteristics were not influenced by amendments doses and no significant differences were observed in leachates composition (control and amended treatments) after 13 months. Amendments application led to differences between the solid phases of the efflorescent salts formed on the surface of the control and the amended treatments. The efflorescent salts contained very soluble Al sulfates, together with alunite-jarosite-group solid phases in amended samples, and copiapite-group solid phases in control. In the core materials (5 to 10 cm in depth), the mineralogy was similar in both control and amended samples. The presence of various stable solid phases from alunite-jarosite-group, such as jarosite and beudantite (mainly in deeper materials), can explain the low concentrations of trace elements in the leachates after thirteen months.

KEYWORDS Alunite-jarosite-group • Arsenic • Copiapite-group • Lead • Percolation leachates

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INTRODUCTION

The intense mining activity in massive sulfides from Iberian Pyrite Belt (IPB) generates different types of wastes. In the São Domingos mine (IPB) in Portugal, large dumps attaining approximately 0.27 Tg, as well as modern and Roman slags and smelting ashes, contain pyrite-rich materials and other sulfides, which produce acid drainage all year round (Álvarez-Valero et al., 2008; Matos, 2004). The production and circulation of acid mine leachates (or acid mine drainage – AMD) is a serious environmental problem in sulfide mine areas due to the extremely acidic conditions (frequently, pH < 1.5) they generate. The very low pH of the leachates promotes trace elements dispersion to adjacent areas, preventing the establishment of vegetation, as well as harming existing vegetation or other organisms that colonise neighbouring areas, thereby decreasing biodiversity (Abreu et al., 2010; Gil Bueno et al., 1990; Pérez-Lopez et al., 2008; Sánchez-España et al., 2005).

The AMD treatment in the IPB has been unsustainable due to its characteristics, namely, extreme acidity and high multitrace elements concentrations (Sáinz et al., 2003). The large surface area of the tailings promotes sulfide oxidation and consequent AMD generation in a manner that is difficult to control. These sulfide-containing tailings also show rill and gully erosion, and lack of vegetation due to the high acidity and low fertility of these waste materials.

Different organic and inorganic wastes, like red gypsum, sugar foam, sewage sludge, biosolids, fly ashes, pig manure, marble wastes, etc., have been evaluated as amendments to improve some physical, chemical and biological properties of mine tailings and contaminated soils (Basta et al., 2001; Forsberg et al., 2008; Pérez-Lopez et al., 2008; Rodríguez-Jordá et al., 2012; Zanuzzi et al., 2009). Some amendments, such as waste compost and sewage sludge, can contain high concentrations of hazardous trace elements that can increase the environmental risk of their application. Therefore, their use should be carefully monitored.

Due to the different chemical behaviour of each trace element and the specific chemical and/or mineralogical composition of the amendments, selective immobilization and redistribution of trace elements in solid phases can be achieved efficiently (Rodríguez-Jordá et al., 2010, 2012).

The application of amendment mixtures composed of several organic/inorganic wastes, instead of using a single type, can be more efficient in the rehabilitation of mining areas that contain high concentrations of several trace elements in tailings and highly contaminated leachates (Kumpiene et al., 2008). These amendment mixtures, which can make a Technosol in an attempt to create different soil functions, can reduce trace element availability and their concentrations in mine waste leachates (Macías, 2004) through the complementary effects of each amendment component. Several industrial or agro-industrial wastes, due to their intrinsic characteristics (high organic matter content and pH, and low concentration of trace elements, etc.), large available quantities and cost effectiveness, can be an economically attractive target to make a Technosol and a strategy for wastes valorisation (Macías, 2004).

The ecological rehabilitation of the São Domingos mine area can be achieved by minimising the leaching element concentrations and/or improving the chemical characteristics (for instance, increase in pH and decrease in salinity) of the leachates by remediation of mine wastes containing sulfides. The

use of amendments can also contribute to plant colonisation and stimulation of biogeochemical processes that promote mine waste treatment and soil remediation (Abreu and Magalhães, 2010). The success of the multielemental contaminated mine areas rehabilitation depends on the combination of the trace elements in tailings and the selection of adequate amendments (Kumpiene et al., 2008).

Leachates from São Domingos and other mines in the IPB present multielemental contamination (Abreu et al., 2008, 2010; Sánchez-España, 2008; Sánchez et al., 2005). However, few studies are available on the effects of amendments on the leaching of cations and anions from sulfide-containing tailings (Pérez-Lopez et al., 2007), and even less for periods of monitoring longer than one year. Therefore studies on the variation over time of the chemical composition of leachates, following amendments addition to mine wastes, are environmentally relevant for future mine rehabilitation projects.

This study aimed to evaluate the effect of two amendment mixtures (30 and 75 Mg/ha) on the availability of trace elements (cations and anions), which occurred at high concentrations in sulfide-containing tailings, over thirteen months. Understanding changes in geochemistry over time is essential in ensuring successful sulfide mine wastes rehabilitation. Examining mineralogical evolution of mine wastes as a consequence of leaching and the attainment of new equilibria were also a goal of this study.

MATERIALS AND METHODS

Site characterization

Sulfide mine wastes were collected from the São Domingos mine area, an abandoned mine located in the IPB and situated in the municipality of Mértola, SE Portugal. Mining activities date from the pre-Roman period with exploitation of Ag, Au and Cu, mainly in the *gossan*. However, modern exploitation (1857–1966) occurred both in the *gossan* and massive sulfides containing Cu, Zn, As and Pb (Quental et al., 2002). The exploitation and extraction processes produced large amounts of different mining wastes, which covered a surface area of around 3.2×10^6 m^2 (Álvarez-Valero et al., 2008). In the Portuguese IPB, São Domingos is a severe case of the environmental impact of mining due to the dimension of the areas affected by AMD and the volume of wastes and tailings (11×10^6 m^3), with high concentrations of hazardous trace elements (Álvarez-Valero et al., 2008; Matos and Martins, 2006). The sampling area is located within the mine area, SW of the open pit, near the former leaching tanks.

Experiment set-up

Composite samples of the sulfide mine wastes (MW), containing mainly crushed pyrite and smelting ashes, were collected in 2009. These materials were chosen due to their large amounts and wide distribution in the mine area, and their potential risk as a source of contamination (Álvarez-Valero et al., 2008; Quental et al., 2002). In addition, the tailings are bared and exposed to severe water erosion.

The amendments applied at 30 or 75 Mg/ha were mixtures containing distinct organic and inorganic wastes from: green agriculture (plant remains + strawberry substrate at 2:3 *m*/*m*) (AgrW), *Arbutus unedo* L. (ArbW) and *Ceratonia siliqua* L. (CerW) fruit spirits distillation; and rockwool used for strawberry crops (RW). Limestone rock wastes (LRW) with a particle size < 2 mm were also used at 55 Mg/ha (except in control) to raise the mine wastes pH to ≈ 4. These organic and inorganic wastes were used due to their physical and chemical characteristics and the fact that they can be easily obtained in large quantities at almost "zero cost" from the vicinity of the mine.

The tailings and amendments were air-dried, mixed manually and potted. Microcosm assays were performed in pots containing around 6 kg of mine waste materials (total fraction). Three treatments were made (four replicates each): control with only mine wastes (MW); and MW + LRW + Amendment composed of AgrW + ArbW + CerW + RW (1:1:1:1) at 30 and 75 Mg/ha. All treatments were kept at 70 % of water-holding capacity under controlled conditions in a greenhouse. The pots were monitored for thirteen months.

Experimental monitoring and sample analyses

Multielemental concentrations of the initial sulfide-containing mine wastes (fraction < 2 mm) and organic/inorganic wastes were analysed by atomic emission spectrometry with induced plasma (ICP-OES) and instrumental neutron activation analysis (INAA) after acid digestion with $HClO_4$ + HNO_3 + HCl + HF in an international accredited laboratory (Actlabs, ISO/IEC 17025, Activation Laboratories, 2011). The fraction < 2 mm was characterized for total concentrations of C and N (analysed by combustion with a Leco analyser), as well as for electrical conductivity (EC) and pH, both in water suspension (1:2.5 *m*/*V*) and extractable P and K (Póvoas and Barral, 1992). Particle size distribution of mine wastes (total fraction) was also determined by sieving.

Mine waste materials (control) or the mixtures of MW and amendments were collected (0 to 5 cm of depth) with a cylindrical probe, from each pot, after collection of the surface efflorescent salts. These solids and the leachates obtained from deionised water percolation through the materials contained in the pots were collected after one, four, seven and thirteen months of incubation. At the end of the experiment (thirteen months), materials from all the treatments were also collected from a depth of around 10 cm.

Only the mine wastes (control) and amended mine wastes collected after one and thirteen months of incubation were air-dried, homogenised, sieved (< 2 mm) and analysed for pH, EC, total concentrations of C and N by the same methodologies referred to above. Total concentration of S was also determined by combustion with a Leco analyser.

To obtain leachates by percolation, the materials in the pots were irrigated with a volume of deionised water that ensured an excess of 10 % of each pot's water holding capacity, and left to percolate for 24 h. The leachates were collected in plastic flasks linked to the bottom of the pot hole by a plastic tube. The leachates were vacuum filtrated (< 0.45 μm), and pH and EC measured. The filtrate was kept at –18 ºC. The leachates were analysed for: Al, Ca, Cu, Fe, K, Mg, Mn, Na, Pb and Zn total concentrations by flame atomic absorption spectrometry; As by graphite furnace atomic absorption

spectrometry; sulfates by ion chromatography; and phosphates by visible spectrophotometry using the molybdenum blue method (USEPA, 1979).

Efflorescent salts were stored, after collection, in hermetic containers at room temperature far from the sunlight. Mineralogical analysis of the efflorescent salts collected after one and thirteen months, as well as of mine waste materials (total fraction) from the end of the experiment, was performed in random powder samples by X-ray diffraction (XRD) using Cu Kα radiation. The XRD analysis was performed in samples separated under a binocular microscope, and selected by colour and general morphological aspects (lustre, habit, etc.). In spite of the methodology used for mineral identification, the samples were always heterogeneous and the solid phases were not easy to identify, owing to the amount of lines obtained in the XRD spectra. The mineralogical analysis considered the elemental composition and the pH of the leachates, the known or foreseen solubilities of the solid phases, as well as the temperature and relative humidity in the greenhouse. The same efflorescent salts were also analysed by Fourier transform infrared spectroscopy (FTIR).

After being analysed by XRD, the efflorescent salt samples and the mine waste materials were mixed with water (solid:water ratio of 0.033:10 and 1:10 (*m*/*V*), respectively), and shaken for 24 hours. The chemical composition of the obtained aqueous solutions with pH < 3 was determined by high resolution inductively coupled plasma mass spectrometry (Actlabs, ISO/IEC 17025, Activation Laboratories, 2011b).

Data analysis

Statistical analysis was performed with the statistical programme SPSS v18.0 for Windows. All data were checked for homogeneity of variance and normality (Shapiro-Wilk test) and, when possible, a one-way ANOVA was applied. Data not satisfying assumptions for ANOVA were analysed non-parametrically using Kruskal-Wallis ANOVA by a rank test. Significant differences ($p < 0.05$) were analysed by a post-hoc Tukey's test. For statistical purposes, the results below the detection limit were assumed as half of the detection limit. Bivariate Pearson correlations were used to correlate the materials and leachates characteristics ($r > 0.95$). Quality control of the analysis was undertaken by analytical replicate samples and laboratory standards at the international accredited laboratory (Activation Laboratories).

RESULTS AND DISCUSSION

Characterization and time evolution of solid materials

Mine wastes are, in general, complex mixtures of heterogeneous materials, but the sulfide wastes collected from the São Domingos area did not show high variability in their chemical characteristics. These mine wastes presented a pH ≈ 2, large EC (7.4 to 7.5 mS/cm) and low fertility (Table 1). The large total carbon concentration in mine wastes was related to the considerable presence of smelting ashes and small pieces of charcoal. Mine wastes presented 54 % of the materials in the fraction > 2 mm, distributed as follows: ≥ 20 mm (7 %); 10–20 mm (10 %); 8–10 mm (5 %); 5–8 mm (11 %); 2–5

mm (21 %). The large amount of coarse materials together with the high total concentrations of trace elements (Table 2) probably contributed to the lack of vegetation, observed in the field, on dumps composed of these types of wastes.

Table 1 Characteristics of the original sulfide mine wastes from the São Domingos mine area and organic/inorganic wastes used as amendments (min – max).

	MW	AgrW	ArbW	CerW	RW
pH (H_2O)	2.1 – 2.3	6.6 – 7.2	4.9	6.1	7.1
EC (mS/cm)	7.4 – 7.5	0.9 – 3.5	1.7	0.2	3.5
Total C (g/kg)	243.8 – 276.1	286.1	442.6	436.7	111.0
Total N (g/kg)	3.7 – 4.8	9.7	8.9	11.1	9.8
Extractable P (g/kg)	< DL	0.3 – 3.1	0.2	0.1	7.9
Extractable K (g/kg)	0.02 – 0.06	0.2 – 6.6	3.6	9.1	0.7

EC: Electrical conductivity; MW: mine wastes; AgrW: Agriculture wastes; ArbW: residue from the liquor distillation of *Arbutus unedo* L. fruit; CerW: residue from liquor distillation of *Ceratonia siliqua* L. fruit; RW: Rockwool: DL: detection limit

Table 2 Total concentrations of trace elements (g/kg Dry weight) in the original mine wastes from the São Domingos mine, composed of crushed pyrite and smelting ashes, and in the organic/inorganic wastes used as amendments (min – max).

	Mine wastes	AgrW	ArbW	CerW	RW
Al	54.8 – 61.2	4.06	1.37	0.6×10^{-3}	53.4
As	1.02 – 1.14	2.3×10^{-3}	1.0×10^{-3}	0.8×10^{-3}	$< 0.5 \times 10^{-3}$
Ca	6.40 – 6.70	22.56	10.30	11.02	139.0
Cu	1.84 – 2.45	44.6×10^{-3}	0.124	9.4×10^{-3}	0.08
Fe	104 – 110	2.96	3.296	0.999	39.90
K	12.8 – 14.3	1.495	> 1.318	0.963	6.80
Mg	1.4 – 1.8	2.369	1.129	> 9.63	42.10
Mn	0.119 – 0.162	0.248	51.2×10^{-3}	43.1×10^{-3}	1.85
Na	4.20 – 4.60	0.310	0.404	0.404	13.10
Pb	8.97 – 13.80	5.0×10^{-3}	3.1×10^{-3}	0.9×10^{-3}	$< 3 \times 10^{-3}$
S	63.6 – 68.0	NA	NA	NA	2.60
Zn	1.08 – 1.19	75.6×10^{-3}	27.3×10^{-3}	18.5×10^{-3}	0.201

MW: mine wastes; AgrW: Agriculture wastes; ArbW: residue from the liquor distillation of *Arbutus unedo* L. fruit; CerW: residue from liquor distillation of *Ceratonia siliqua* L. fruit; RW: Rockwool; NA: non analysed

Concentrations of As, Cu, Pb and Zn in the initial mine materials were in the same range as those reported by Álvarez-Valero et al. (2008) for pyrite-rich samples and smelting ashes from São

Domingos. The physical and chemical characteristics of the mine wastes were associated with their predominant composition – materials containing sulfides, potential AMD generators.

All the organic and inorganic wastes used as amendments presented chemical characteristics considered beneficial and safe for land application and rehabilitation of mine wastes. Concentrations of total nitrogen and extractable phosphorus and potassium, in addition to pH values, were larger than those in mine wastes (Table 1), while the lowest EC was observed (0.2 to 3.5 mS/cm) in all the amendments. Wastes from spirits distillation (ArbW and CerW) showed the highest total carbon concentrations (436.7 to 442.6 g/kg). Concentrations of trace elements in the amendments were much lower than those in mine wastes (Table 2).

After one month of incubation and at the end of the experiment (thirteen months of incubation), amendments addition improved the structure of the materials by increasing the organic matter content and consequently raising the water-holding capacity. The amendments reduced EC by 31 to 53 % compared to control (Table 3). The pH of the amended mine wastes increased to 3.5–3.6 and ~2.1 after one and thirteen months of incubation, respectively (Table 3) despite the presence of limestone rock wastes (in the initial composition of the amendments. However, when materials/water suspensions (1:10 *m*/*V*) from the first month of incubation were shaken for one and five days at room temperature, the measured pH were ~2.2 for control (for both shaking times) and 4.2-4.5 and 5.3-7.0 for one and five days, respectively, for the amended samples. Similar shaking experiments performed with materials after four months of incubation presented pH values ($2.1 < pH < 2.5$) in the same range as that of the collected leachate percolations (Fig. 1). Zanuzzi et al. (2009) in another microcosms assay involving the Brunita mine tailings pond, observed an increase in the pH of the materials following the addition of amendments, even after 24 months, from 2.8 (treatment not amended) to 7.3 (application of marble wastes + pig manure or marble wastes + sewage sludge). However, those authors applied larger doses (166.7 Mg/ha) of marble wastes with larger particles (< 2 mm: 26 %; 2–5 mm: 74 %), compared to the present study (55 Mg/ha, < 2 mm: 100 %). The comparison of the pH values gotten in this work, for the shaken materials/water suspensions, with the results of Zanuzzi et al. (2009) show that the presence of limestone in the amendments increases the pH to values higher than four. Thus, the pH lower than four, measured in this work, in the leachates from the amended materials, after one month of incubation, indicate that the limestone could not be in contact with the surrounding aqueous medium. This can be explained by the formation of a layer of calcium sulfate on the surface of the limestone grains. The Ca sulfate is the product of the reaction between the calcium carbonate and the sulfate ion present in the acid solutions generated by the pyrite oxidation. The calcium sulfate surface layer was dispersed after shaking, promoting the contact between the limestone and the aqueous solution, increasing the pH of the materials/water suspensions. However, after the fourth month of incubation the limestone was already dissolved once there was no change in the pH of the shaken materials/water suspensions. The decrease in the limestone effect at the beginning of the incubation, due to its reaction with the generated acidic leachates, was favoured by the small grain size (< 2 mm) of the used material, which was therefore one disadvantage of this amendment. In fact, the kinetics of the limestone reaction increases when the size of the solid particles decreases. During the entire experiment, the acidity of mine wastes increased in all

treatments, with and without amendments (Table 3). The amendments, at both doses (30 and 75 Mg/ha), were not efficient in AMD pH buffering.

Table 3 Characteristics of mine wastes samples from different treatments, collected after one month of incubation and at the end of the experiment (Mean ± SD; *n* = 4).

	Control	**Treatment with amendments**	
		30 Mg/ha	**75 Mg/ha**
	After one month of incubation		
pH (H_2O)	2.5 ± 0.1^{bA}	3.5 ± 0.03^{aA}	3.6 ± 0.1^{aA}
EC (mS/cm)	8.4 ± 1.5^{aA}	4.0 ± 1.0^{bB}	4.3 ± 0.3^{bB}
Total C (g/kg)	208.0 ± 22.0^{aA}	182.0 ± 19.9^{aA}	204.5 ± 18.0^{aA}
Total N (g/kg)	4.1 ± 0.4^{aA}	3.7 ± 0.4^{aA}	4.3 ± 0.5^{aA}
Total S (g/kg)	52.8 ± 2.2^{aA}	51.1 ± 2.1^{abA}	46.7 ± 3.7^{bA}
	After thirteen months of incubation		
pH (H_2O)	1.6 ± 0.1^{bB}	2.1 ± 0.1^{aB}	2.1 ± 0.2^{aB}
EC (mS/cm)	10.9 ± 1.1^{aA}	7.0 ± 1.3^{bA}	7.5 ± 2.2^{bA}
Total C (g/kg)	173.3 ± 7.5^{aB}	154.3 ±11.6^{aA}	145.5 ± 2.9^{aB}
Total N (g/kg)	3.3 ± 0.1^{aB}	3.1 ± 0.1^{aA}	3.1 ± 0.1^{aB}
Total S (g/kg)	35.7 ± 1.3^{bB}	48.7 ±0.5^{aA}	49.9 ± 1.7^{aA}

EC: Electrical conductivity
Data of the same characteristic followed by a different letter are significantly different (p < 0.05). Small letters indicate comparisons between treatments from the same sampling period and capital letters indicate comparisons between the same treatments from different sampling periods

After thirteen months of incubation, total sulfur concentration in the mine wastes with amendments was larger than that of control (Table 3). A significant reduction of total sulfur was only observed in the control (32 %) between one and thirteen months. Efflorescent salts formed crusts on the surfaces of the controls, reaching 5 mm in thickness. On the amended surfaces, there were only a few efflorescent salts. Both surfaces displayed efflorescent salts containing mixtures of soluble sulfates, as described in Section 3.3. Besides the migration of sulfates to the surface, leachates collected from control contained higher concentrations of sulfates (112 g SO_4/L in leachates collected in the fourth month) than those that were treated with amendments (≈ 23 g SO_4/L in leachates collected also in the fourth month).

Characterization and time evolution of leachates

Electrical conductivity and pH

During the experimental period, pH and EC of the leachates from the treatments with amendments (pH: 1.95–2.47; EC: 4.36–21.05 mS/cm) were significantly different from those of the control (pH: 1.13–1.58; EC: 11.08–51.92 mS/cm), although both were acidic and with high EC (Fig. 1). The

amendments increased the pH of leachates by more than 0.5 units and reduced the EC by more than 43 %. These values contrast with the results of Ciccu et al. (2001) and Pérez-López et al. (2007), who applied fly ashes to pyritic sludge or to soils contaminated by reddish tailings and observed an increase of more than two units for the pH of the leachates (from 2 to 7.5 and from 6 to 8, respectively) after 210 and 350 days, respectively. However, EC was only reduced in the experiments with pyritic sludge (0.30 mS/cm). Rodríguez-Jordá et al. (2012) also recorded an increase of the pH of the leachates (from 3.4 to values > 6) from São Domingos mine sediments 40 days after application of different calcium carbonate-containing amendments (sugar foam or mixtures of sugar foam + ashes from combustion of biomass or sugar foam + red gypsum + ashes from combustion of biomass). The pH values obtained by Rodríguez-Jordá et al. (2012) confirm our previous statement that the pH of amended wastes and their leachates is higher than four when calcium carbonate is present.

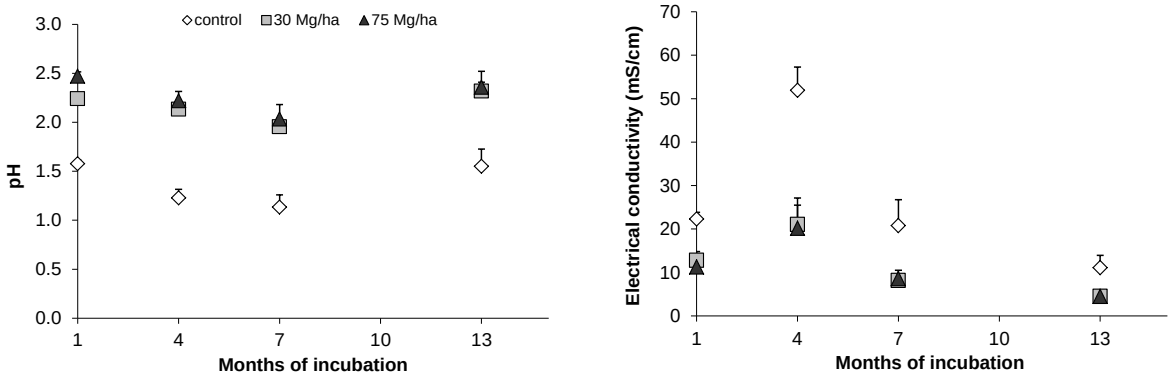

Fig. 1 Variation, with time, of pH and electrical conductivity in leachates, obtained by percolation, from sulfide mine wastes without and with amendments application at 30 and 75 Mg/ha (Mean ± SD; *n* = 4).

The pH of the leachates from control was lower and the EC values higher than those measured in the leachates from pyritic sludge by Pérez-López et al. (2007, pH ≈ 2; EC: 2–4 mS/cm). However, the present work results were in the same range as those reported for acid mine drainages in the IPB (Abreu et al., 2010; Sanchez-España et al., 2005, 2008).

The large quantity of sulfides in the mine wastes used in the present study can be one of the possible explanations for the great differences observed in leachates pH between this work data and those obtained by Forsberg et al. (2008) for Aitik Cu mine tailings containing only traces of metal sulfides whose pH ranged between 3.5 and 7.5.

Until the seventh month of incubation, the decrease of the leachates pH was observed in all the treatments. However, pH values at the end of the experiment (thirteen months) were similar to those of the first month of incubation. This represented a pH increase, between the seventh and thirteenth month of incubation, from 1.15 to 1.55 for control, and from ≈ 1.9 to ≈ 2.3 for amendment-treated samples. The decrease of the pH of the leachates during an experimental period of 630 days was also observed by Forsberg et al. (2008) in the Aitik Cu mine tailings leaching.

Regarding EC, the control samples exhibited a different trend compared to amended ones. Control EC values were in the same range as those reported for AMD from some IPB mines (Abreu et al., 2010; Sanchez-España et al., 2005, 2008). Control showed significant variations in EC, with a 2.5–fold

increase in the fourth month (from 22.30 after the first month to 51.93 mS/cm), and a decrease to 20.78 mS/cm in the seventh month and 11.08 mS/cm in the last month. In the leachates from the control, the highest values of EC (Fig. 1) were measured in the fourth month, which agrees with the majority of the elemental total concentrations measured by other techniques. In treatments with amendments, the values of EC in the first and fourth months of incubation (11.3 and 21.1 mS/cm, respectively) were not significantly different, but in the following months, a significant and progressive decrease was observed, reaching a value of around 4 mS/cm in the thirteenth month. The sampling made after one month of incubation occurred in spring, while the fourth and seventh month samplings occurred in summer. During spring and summer, the strong evaporation contributed to continuous crust formation of salts crystallising on the materials' surface. Irrigation usually caused complete dissolution of the efflorescent salts, but this was not complete during the warmest months, increasing the thickness of the surface crusts. The rise in the amount of surface salts could have contributed to the EC reduction observed during the experiment in all the treatments, as a result of a decrease in the concentrations of dissolved ions.

In terms of the possible biogeochemical rehabilitation effect, the increase of the amendment doses from 30 to 75 Mg/ha did not significantly modify the leachates characteristics originated by the waste materials. The environmental impact of the mine wastes leachates is still very high.

Anions and cations concentrations

The water in contact with the mine wastes promoted the oxidation of the sulfide minerals, resulting in the formation of acid solutions and the consequent leaching and release of high amounts of cations, and anions containing mainly As and S. The leachates collected from control presented a typical multielemental AMD composition (Figs. 2 and 3) from the IPB (taking in account both seasonal and mine variability) (Abreu et al., 2008, 2010; Sánchez-España et al., 2005, 2008) and the Valdarcas mine in the north of Portugal (Valente and Gomes, 2009).

Total concentrations of anions and cations in leachates were not related to their total concentrations in the mine wastes. However, the very low concentrations of phosphates (Fig. 2) in the leachates collected from the control suggested a low total concentration of these ions in mine wastes ($[phosphate]_{total}$ = 1.01 g PO_4/kg). No significant variations in phosphate concentrations were observed with time in the control (Fig. 2, 1.35–17.82 mg PO_4/L).

The amendments contributed to a significant increase in the phosphate total concentrations in the leachates (15.23–98.25 mg PO_4/L), compared to the control (1.35–17.82 mg PO_4/L), at least for the first three sampling periods (Fig. 2). The phosphates in the amendments (Table 1) were linked to the organic matter and nutrient solution incorporated into the rockwool, which came from strawberry cultivation in greenhouses. The amendments maintained leachate phosphate levels between 69.04 and 98.25 mg PO_4/L, until the seventh month of incubation. A significant reduction of more than 63 % was observed in the phosphate concentration (15.23 and 30.96 mg PO_4/L for 30 and 75 Mg/ha, respectively) after the thirteenth month. The crystallisation of Ca-containing phosphates in the

efflorescent salts and other metal phosphates in the core materials (Section 3.3) could explain this decrease in phosphate concentrations in the leachates.

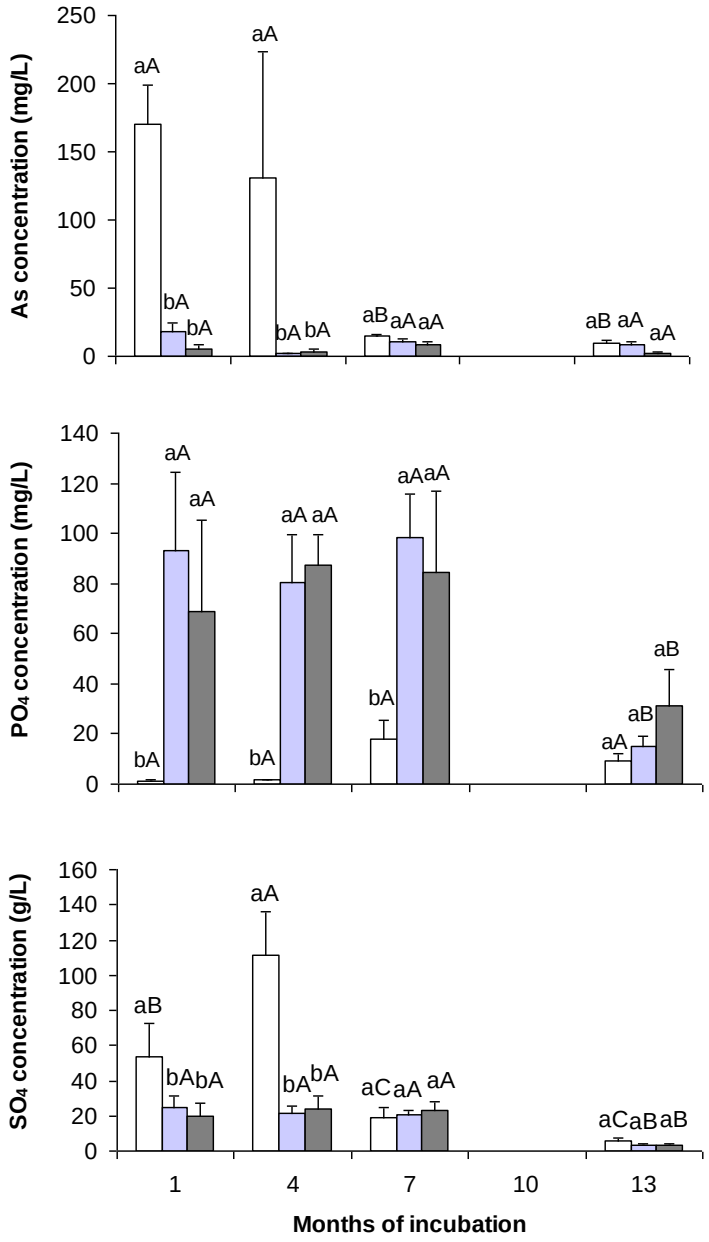

Fig. 2 Variation, with time, of anion concentrations in leachates, obtained by percolation, from sulfide mine wastes without and with amendments application at 30 and 75 Mg/ha (Mean ± SD; *n* = 4). Data followed by a different letter are significantly different ($p < 0.05$). Small letters indicate comparisons of different treatments from the same sampling period and capital letters indicate comparisons of each treatment with time.

A considerable decrease in the concentration of some elements in leachates was observed following treatment with amendments, in comparison with control samples leachates (Figs. 2 and 3). The most extreme decrease, in the first month, was observed for As, which diminished by 94 % followed by Fe and Pb, whose total concentrations decreased by approximately 88 and 71 %,

respectively. Sulfate and Cu concentrations decreased by around 56 %, while Al, Mn and Zn concentrations were reduced by 31, 30 and 25 %, respectively.

The total concentrations of Al, As , Cu , Fe , Pb , Zn and sulfate in the leachates from control samples were significantly higher than those in the leachates from amended ones in the fourth month of incubation (Figs. 2 and 3). In the leachates from control, the concentrations of Al, Cu, Pb, Zn and sulfate decreased significantly in the seventh and last months of the experiment (Figs. 2 and 3). Decrease of the total concentrations of Cu, Fe, Pb, Mn and Zn in the leachates was also obtained with fly ashes or bone meal application to pyritic sludge or soils contaminated with reddish tailings (Ciccus et al., 2001; Pérez-López et al., 2007; Sneddon et al., 2006). Rodríguez-Jordá et al. (2012) also observed a decrease of the total concentrations of Cu and Zn in the leachates from São Domingos mine sediments after applying sugar foam-containing amendments. However, these authors noted an increase in the total concentrations of As and Pb in leachates after amendments application, which are in contrast to the results of the present study.

The total concentrations of As and Fe in the leachates from amended samples showed no significant variations with time. There was no noticeable decrease in the first month, in the concentrations of Ca, K and Na in these leachates when compared to control, with Na and K exhibiting very low concentrations in the leachates from amended samples all the time (Fig. 3). However, Ciccus et al. (2001) and Pérez-López et al. (2007) observed an increase in the total concentrations of Ca, K and Na in leachates from amended treatments.

The concentration of Mg in the leachates from control was lower than that in the leachates from samples treated with 75 Mg/ha of amendments in the first month, due to the fact that the amendments had high total concentration of Mg (Table 2 and Fig. 3).

With the exception of As and K, whose highest concentrations in leachates from control samples were found in the first and seventh months, respectively, the highest concentrations of Al, Ca, Cu, Fe, Mg, Mn, Na, Pb, Zn and sulfate were measured in the fourth month (Figs. 2 and 3). At the end of the experiment, the total concentrations of Al, As, Cu, Fe, Mg, Mn, Zn and sulfate in the leachates from control matched their concentrations in the leachates from the amended samples. In opposition to the present work results, no regular pattern was reported by Forsberg et al. (2008) in the concentrations of Al, Cu, Mn, Pb and Zn in leachates from Aitik mine tailings; however, they did note a progressive increase in their concentrations with time.

The total concentrations of K, Na and Pb in the leachates from control were always significantly higher than those in the leachates from amended samples, even at the end of the experiment (Fig. 3).

Figures 2 and 3 show that the increasing the dose of amendments did not significantly affect the multielemental composition of the leachates. The variations in the leachates pH with time did not correlate with concentrations of anions and cations in all the treatments.

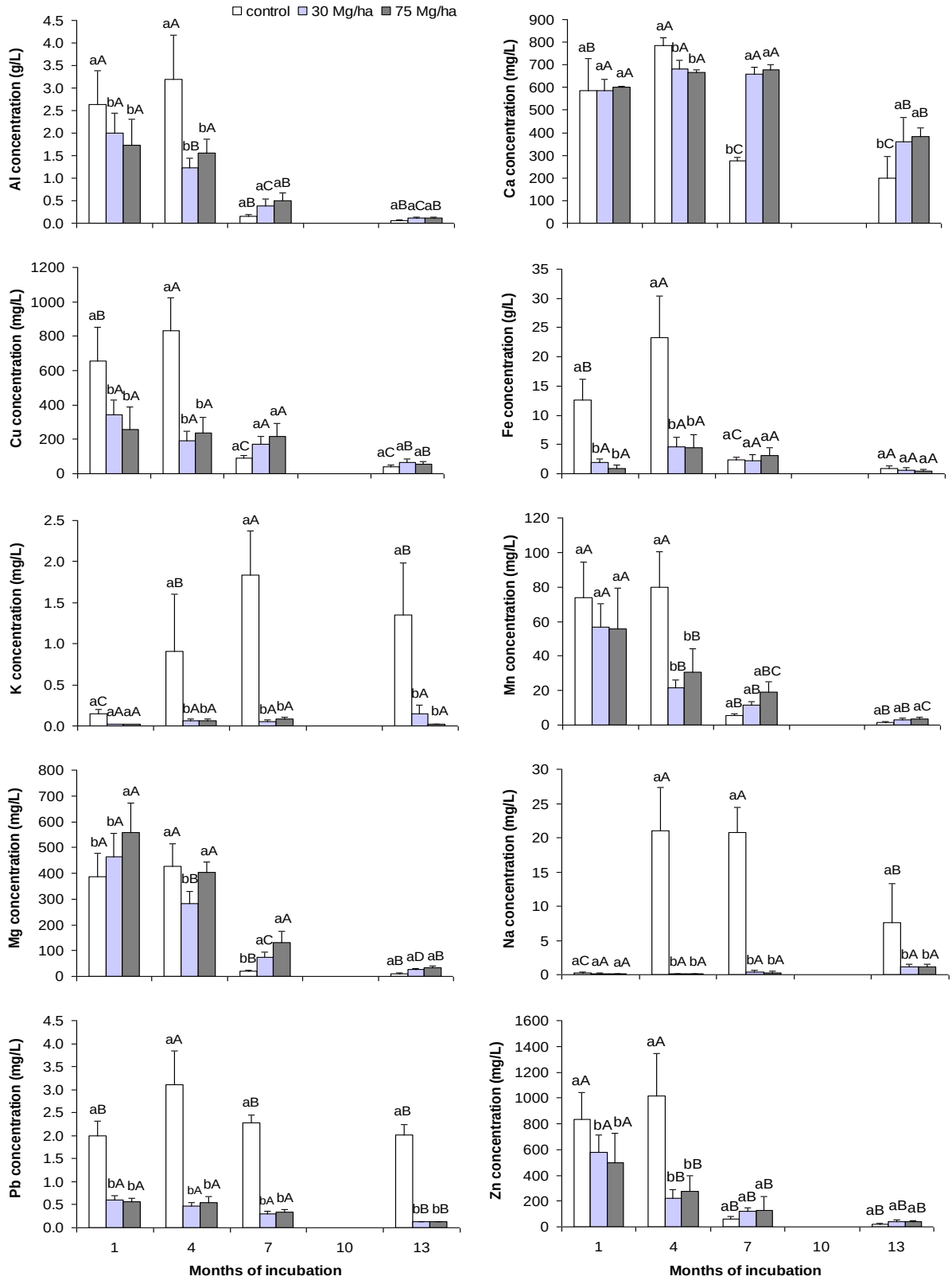

Fig. 3 Variation, with time, of cation concentrations in leachates, obtained by percolation, from sulfide mine wastes without and with amendments application at 30 and 75 Mg/ha (Mean ± SD; n = 4). Data followed by a different letter are significantly different (p < 0.05). Small letters indicate comparisons of different treatments from the same sampling period and capital letters indicate comparisons of each treatment with time.

In general, concentrations of cations in leachates were variable and difficult to predict and explain, due to the complexity of solid-phase trace elements composition and concentrations, aqueous solution characteristics, rates of acid generation relative to neutralisation, and the possible existence of various physico-chemical conditions (Sullivan and Yelton, 1988). In the present work, a decrease in the concentrations of the hazardous chemical elements was evident with time, but Pérez-López et al. (2007) found an increase in Pb concentration from 3 to 35 mg/L in leachates from pyritic sludge. Rodríguez-Jordá et al. (2012) also observed an increase in As concentrations, with time in leachates from São Domingos sediments amended with sugar foam + biomass ash. Cao et al. (2003), Forsberg et al. (2008), Pérez-López et al. (2007) and Rodríguez-Jordá et al. (2012) reported, as in the present work, decreased concentrations of several chemical elements in leachates following the application of amendments. However, Forsberg et al. (2008) reported that adding sewage sludge to mine tailings from Aitik copper mine did not lead to the immobilisation of Al, Cu, Mn and Pb during all leaching cycles. The different results obtained with different amendments could be attributed to the elemental chemical composition as well as to the physico-chemical properties of the amendments and substratum (soils and/or mine wastes) (McBride, 2003). Systems with different chemical compositions promote different behaviours.

The amendments obviously interact with the mine waste materials. For example, limestone reacts with the acid generating media and also organic matter. In spite of the small increase in pH of mine waste leachates following amendments application, interactions between the mine wastes and amendments can be perceived. The experiments discussed in Section 3.1 show that limestone was not present in the amended materials in the fourth month of incubation. On the other hand, the organic matter of the amendments can be oxidized, originating weak organic acids with some buffering capacity. The small increase in pH of the leachates from amended mine wastes, when compared to control, can be explained by the action of those organic acids. The organic matter can also inhibit the possible oxidation the sulfide minerals. Residues of crystalline organic compounds were proposed in the preliminary computer analysis of the X-ray diffractograms of the amended wastes, described in Section 3.3. The decrease of the total concentrations of As and sulfate in the leachates from amended samples following the addition of organic matter is not easy to explain by surface adsorption mechanisms, since the presence of mainly negatively charged organic molecules or ions will not attract the negatively charged As or S containing ions. Hydronation of organic molecules can occur at low pH, but As ions are also hydronated into neutral species, diminishing the possibility of surface adsorption. Fitz and Wenzel (2002) stated that they did not see evidence of the organic matter contribution to the sorption of significant amounts of As in soils. The same mechanism of hydronation of organic groups neutralising their negative charges or even, at very low pH, becoming positively charged could explain the possible lack of sorption of the positive metal ions in very acidic media. Besides the sorption mechanisms, ions can be immobilised by the formation of insoluble secondary solid phases (metal arsenates) (Abreu et al., 2009; Kabata-Pendias and Pendias, 2001; Kumpiene et al., 2008; Santos et al., 2012). The decrease of the total concentrations of Al, Cu, Fe, K, Mn, Na, Pb and Zn in the leachates from samples treated with both doses of amendments, compared to control, is also well explained by the crystallisation of solid phases.

During thirteen months of incubation, the sulfide materials (Table 2) from control lost cumulatively in the leachates, 4.8 % of their total concentration of S and ~1.7 % of their total concentrations of As and Fe (Figs. 2 and 3). However, after amendments application, the fraction of these elements lost by leaching from the sulfide materials decreased significantly to about 2.5 % of the total concentration of S and less than 0.5 % for As and Fe.

Mineralogical characterization

The maintenance of the water-holding capacity during the experimental periodkept the core materials continuously wet in an acidic media, promoting the dissolution of some solid phases and the crystallisation of new ones with compositions different from those of the parent materials. The contact of the constantly moist mine waste with atmospheric oxygen promoted the oxidation of sulfide minerals, the decrease of the pH of the leachates, minerals dissolution, and chemical elements leaching. Hazardous chemical elements can be retained by the formation of secondary solid phases or become available depending on the composition of the leaching solutions. Hazardous chemical elements retention in the solid phases can be explained by the crystallisation of metal arsenates, phosphates and sulfates. The most soluble solids, under specific chemical and physical conditions, crystallise at the surface as efflorescent salts, while the less soluble solids crystallise in the core materials contained in the pots. However, the solubility of arsenates and phosphates strongly depends on pH as a result of the hydrolysis of the anions and consequent displacement of the chemical equilibria related to the dissolution processes. The very acidic aqueous solutions have very high leaching capacities and can dissolve the solid arsenates and phosphates with low solubility in almost neutral aqueous solutions. These solutions move to the surface by the capillarity phenomena and the very soluble solid phases then crystallise due to water evaporation.

Efflorescent salts

The formation of efflorescent soluble salts on the materials of the surface of each pot began when the atmospheric temperature increased and air humidity decreased, promoting dryness of the surface, in spite of the constant watering to meet the water-holding capacity of the materials inside the pots. The thickness of the surface crust was higher in the control than in the amended samples as a consequence of the higher ionic concentrations in the control pore aqueous solution. In all pots, the thickness of the surface crusts increased during the hottest months, undergoing partial re-dissolution during irrigation. Under similar atmospheric conditions, in the Iberian Pyrite Belt mining areas, very soluble crusts are formed on the surfaces of mine wastes, leaching lagoon banks and beds, and river beds (Abreu et al., 2010; Romero et al., 2006). However, the first rainfalls completely dissolve the efflorescent salts, leading to increased levels of trace elements in the mine drainage water (Abreu et al., 2010; Álvarez-Valero et al., 2007; Nieto et al., 2007; Sánchez-España, 2008; Sánchez-España et al., 2005).

Different efflorescent salts were identified depending on the pH and other ionic composition of the crystallisation medium, presence or absence of organic matter, and the duration of contact between the water and solid phases. Air temperature and humidity can also influence the composition of the efflorescent solid phases.

After one month of incubation, the efflorescent salts of the control were identified as the secondary new phases aluminium sulfates ($Al_2(SO_4)_3$•16-17H_2O), aluminocopiapite ($(Al_{2/3x},Fe_{1-x}^{2+},V_{1/3x})(Al_x,Fe_{1-x}^{3+})_4(SO_4)_6(OH)_2$•20$H_2O$) and aluminocoquimbite ($(Al_x,Fe1\text{-}x)_2(SO_4)_3$•9$H_2O$) with different degrees of substitution of Al in the Fe positions (Fig. 4). The data presented in Table 4, obtained from the dissolution of the efflorescent salts, show that these solid phases must contain mainly Al and Fe. Some of the other chemical elements presented in the Table 4 can be in the lattice of the reported solid phases in trace amounts. Other solid phases must also be present but their tiny amounts did not allow their identification by X-ray diffraction.

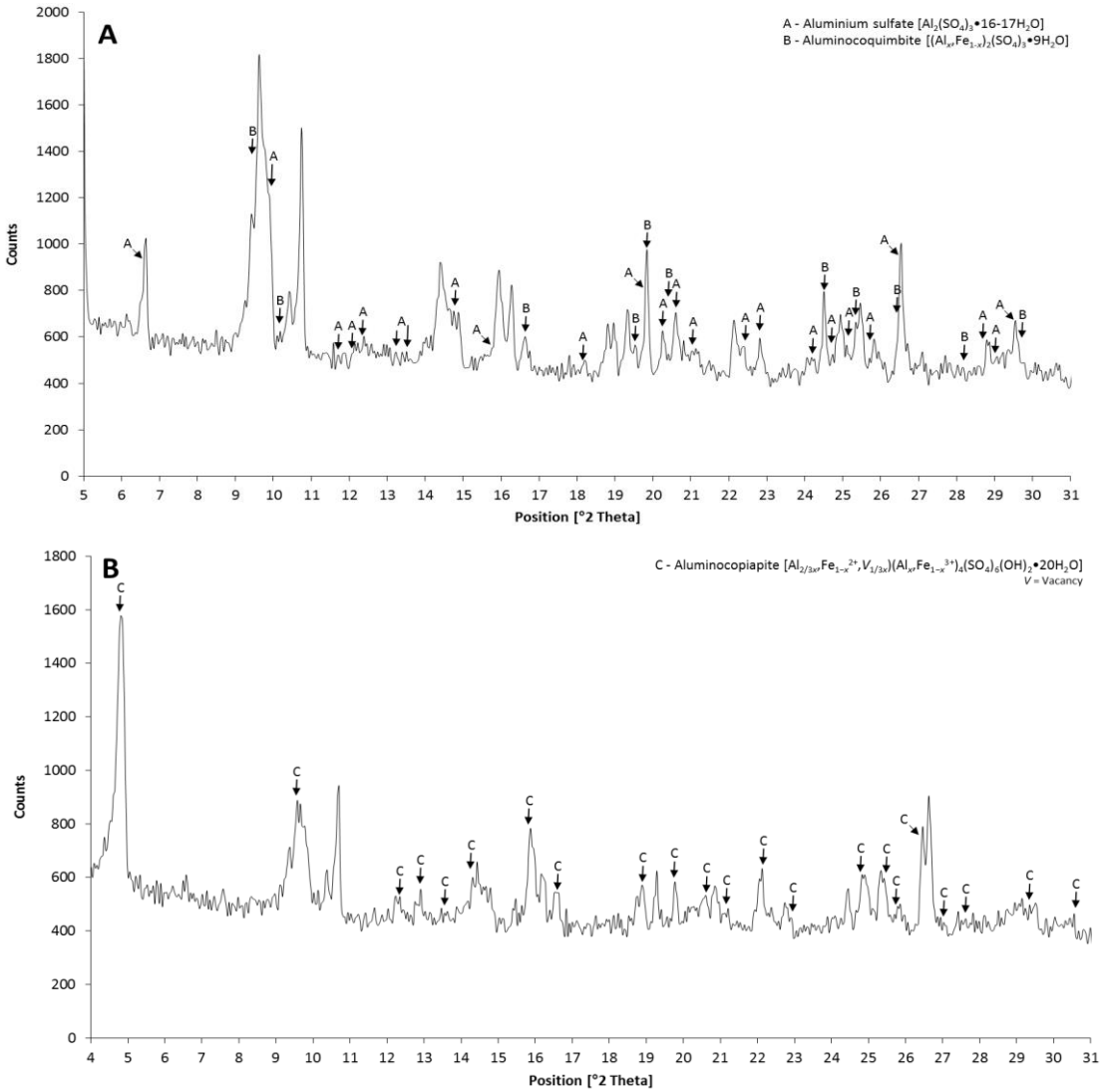

Fig. 4 X-ray diffractograms of two samples (A and B) of the surface efflorescent salts identified in control collected after one month of incubation.

Table 4 Multielemental composition of surface efflorescent salts from sulfide mine wastes without (control) and with amendments application at 30 and 75 Mg/ha.

Samples	Al (mg/g)	As (µg/g)	Ca (mg/g)	Cu (mg/g)	Fe (mg/g)	K (µg/g)	Mg (mg/g)	Mn (mg/g)	Na (µg/g)	Pb (µg/g)	Zn (mg/g)
After one month of incubation											
Control – A*	14.96	80.71	0.86	1.26	58.16	210.27	0.46	0.15	108.39	468.84	3.12
Control – B*	4.06	78.18	0.58	0.25	9.42	156.38	1.65	0.19	237.69	444.23	1.13
Amended at 30 Mg/ha – A	31.20	1.85	2.94	5.68	4.04	33.20	6.28	1.13	73.00	0.55	13.02
Amended at 75 Mg/ha – A*	29.00	1.83	27.64	4.47	2.46	73.71	7.43	1.14	101.08	0.25	10.84
Amended at 75 Mg/ha – B*	44.51	4.42	3.53	5.66	3.84	130.35	10.49	1.61	84.68	0.19	15.20
After thirteen months of incubation											
Control – A*	1.84	154.61	0.65	0.43	0.31	197.22	0.17	0.19	93.28	362.76	1.28
Control – B*	5.68	294.17	0.93	1.67	45.44	381.55	0.58	0.19	323.30	675.73	2.27
Amended at 30 Mg/ha – A	10.21	109.30	2.28	5.52	46.60	368.17	1.35	0.26	284.18	871.52	3.83
Amended at 30 Mg/ha – B	6.16	22.79	1.43	3.96	19.24	612.75	1.34	0.28	77.91	3.66	3.74
Amended at 75 Mg/ha – A*	8.07	43.57	1.54	5.06	17.72	51.23	2.04	0.34	78.31	0.58	4.27
Amended at 75 Mg/ha – B*	15.41	33.62	145.01	3.11	23.99	54.70	1.85	0.30	96.01	2.12	3.05
Amended at 75 Mg/ha – C*	7.58	1.36	6.27	2.21	2.68	114.21	1.20	0.20	144.67	1.33	2.31

*Samples indicated with asterisk correspond to salts analysed by DRX.
The determination of multielemental composition of other salts from amended treatment at 30 Mg/ha, after one month of incubation, was not possible due to insufficient sample.

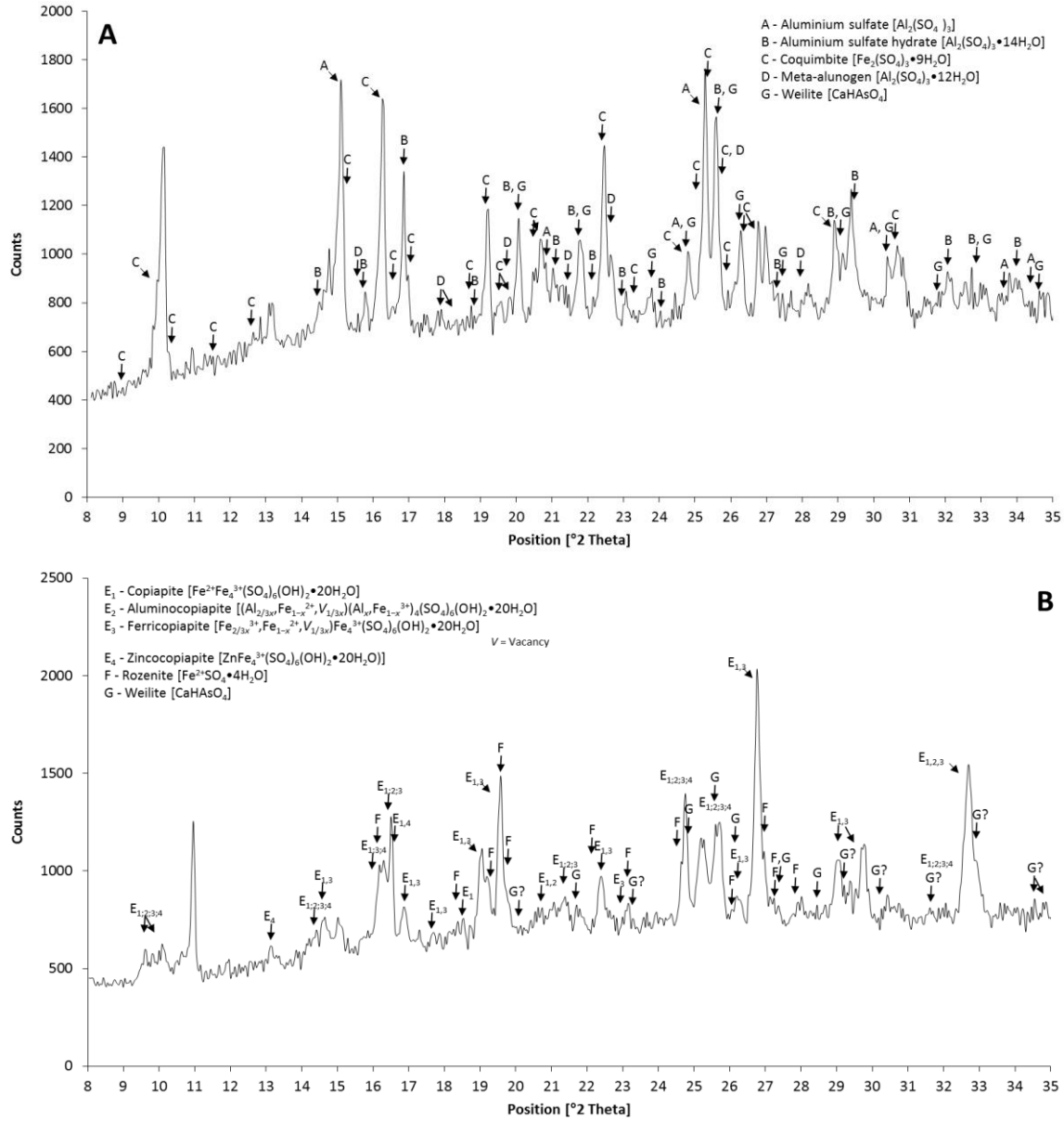

Fig. 5 X-ray diffractograms of two samples (A and B) of the surface efflorescent salts identified in control collected after thirteen months of incubation.

After thirteen months of incubation, a higher diversity of soluble sulfates was identified among other solids (quartz, weilite ($CaHAsO_4$), metal sulfides, etc.) (Fig.5). The existence of high total concentrations of S, Al and Fe ions in the mine waste materials (Table 2) produced sulfates with different chemical composition and structure, such as aluminium sulfate ($Al_2(SO_4)_3$), meta-alunogen ($Al_2(SO_4)_3$•$12H_2O$), tamarugite ($NaAl(SO_4)_2$•$6H_2O$), coquimbite ($Fe_2(SO_4)_3$•$9H_2O$), several solid phases or solid solutions (with different degrees of metal ions substitution) of the copiapite-group [aluminocopiapite, copiapite ($Fe^{2+}Fe_4^{3+}(SO_4)_6(OH)_2$•$20H_2O$), ferricopiapite ($(Fe_{2/3x}^{3+},Fe_{1-x}^{2+},V_{1/3x})Fe_4^{3+}(SO_4)_6(OH)_2$•$20H_2O$) and zincocopiapite ($ZnFe_4^{3+}(SO_4)_6(OH)_2$•$20H_2O$)], rozenite ($Fe^{2+}SO_4$•$4H_2O$) and probably poitevinite ($(Cu,Fe^{2+})SO_4$•H_2O) (Fig. 5). The slight increase of the concentration of several ions in the aqueous solutions, obtained from the dissolution of the efflorescent salts, in relation to the values obtained after one month of incubation (Table 4) explains the variety of the solid solutions identified by XRD. On the other hand, the changes in the

environmental humidity and temperature during the elapsed time of the experiment (13 months) justify the existence of solid phases with similar composition but different degrees of hydration. Some of these minerals, e.g., coquimbite, aluminocopiapite, ferricopiapite, rozenite and poitevinite, were also reported in mine wastes from São Domingos (Abreu et al., 2010; Álvarez-Valero et al., 2007; Sánchez-España, 2008), other IPB mines and the Valdarcas mine (Romero et al., 2006; Sánchez-España, 2008; Sánchez-España et al., 2005; Valente and Gomes, 2009). Copiapite-group minerals are common in the extremely acid environments of sulfide mine areas, where associations with iron sulfates such as melanterite and coquimbite are frequent (Jamison et al., 2005a; Romero et al., 2006). The pH values of the waste materials in the control (Table 3) in the first and thirteenth months, coupled with the pH values of the leachates collected from control (Fig. 1), indicate that all the solid phases crystallised on the materials of the surface of the control samples are stable under extremely acidic conditions (pH < 1.5), as the abovementioned field data also corroborate (Abreu et al., 2010). The pH of the pore waters at the surface of the materials in the control decreased as the superficial water evaporated, increasing the concentrations of the dissolved ions and promoting the crystallisation of very soluble salts.

In the efflorescent secondary salts following amendments treatments (30 and 75 Mg/ha) after one month of incubation, mainly aluminium-, copper- and iron-containing sulfates, such as alum-K ($KAl(SO_4)_2•12H_2O$), alunogen ($Al_2(SO_4)_3•17H_2O$), beaverite ($Pb(Cu^{2+},Fe^{3+})_3(SO_4)_2(OH,H_2O)_6$), halotrichite ($Fe^{2+}Al_2(SO_4)_4•22H_2O$), linarite ($Cu^{2+}Pb(SO_4)(OH)_2$) and melanterite ($Fe^{2+}SO_4•7H_2O$), were identified (Fig. 6). The chemical composition of the minerals agrees with the data presented in Table4.

After thirteen months of incubation, arsenate, oxalate, phosphate and sulfate minerals were identified in the efflorescent salts crystallising on the amended materials (Fig. 7): several minerals from the alunite-jarosite-group [beaverite, beudantite ($Pb(Fe^{3+})_3(AsO_4)(SO_4)(OH)_6$), corkite ($Pb(Fe^{3+})_3(PO_4)(SO_4)(OH)_6$), (hydronium-, natro-) jarosite ($(H_3O,K,Na)(Fe^{3+})_3(SO_4)_2(OH)_6$), plumbojarosite ($Pb(Fe^{3+})_6(SO_4)_4(OH)_{12}$), chenite ($Cu^{2+}Pb_4(SO_4)_2(OH)_6$)], epsomite ($MgSO_4•7H_2O$), gypsum ($CaSO_4•2H_2O$), humboldtine ($Fe^{2+}C_2O_4•2H2O$), moolooite ($Cu^{2+}C_2O_4•nH_2O$ ($0 < n < 1$)) and weilite ($Ca(HAsO_4)$). Once more, the chemical composition of the minerals agrees with the data presented in Table 4. The existence of solid phases containing sulfates was confirmed by infrared (Moenke, 1962; Mindat, 2013), as shown in the spectra of figure 8.

Alunogen, alunite-jarosite-group minerals, epsomite, gypsum and melanterite were also reported in Valdarcas, São Domingos and other mine areas from IPB at sites with pH > 1.5 (Abreu et al., 2010; Álvarez-Valero et al., 2008; Matos and Martins, 2006; Rosado et al., 2008; Romero et al., 2006; Sánchez-España, 2008; Sánchez-España et al., 2005; Valente and Gomes, 2009). Halotrichite was reported in the mining areas and river banks with pH values of 1.5 to 2.5 (Sánchez-España, 2008; Sánchez-España et al., 2005; Valente and Gomes, 2009). Gypsum is found in the referred mines often associated with copiapite and jarosite. Jamison et al. (2005b) observed that jarosite was frequently found in mine wastes environments with pH > 2, but not in mine areas with pH < 1.

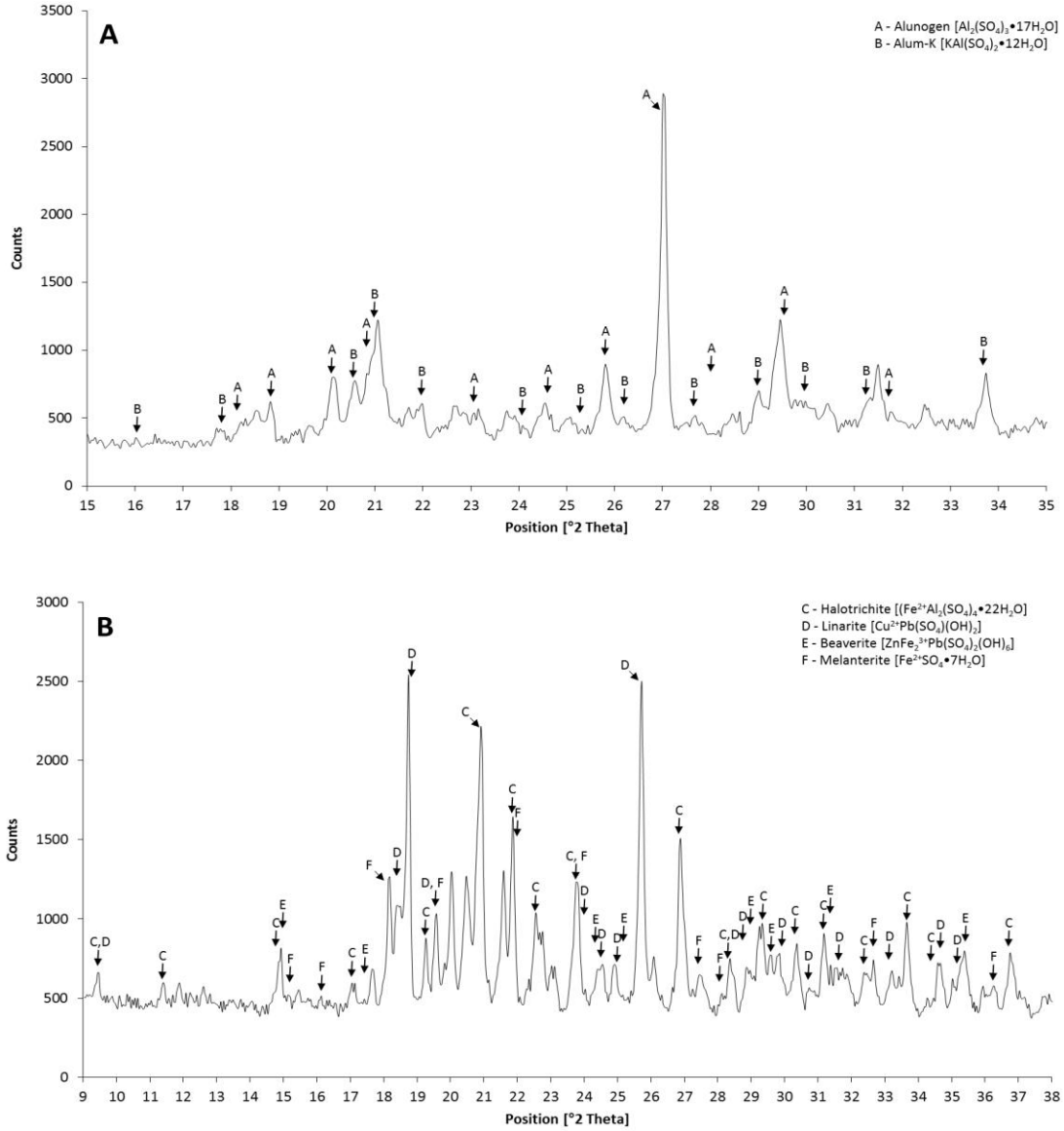

Fig. 6 X-ray diffractograms of two samples (A and B) of the surface efflorescent salts identified in amended materials collected after one month of incubation.

The organic matter included in the amendments can retain more water and allow the formation of more hydrated phases than the meta-alunogen and rozenite identified in the efflorescent salts from control. This is due to the increase in water activity, which consequently raises the pH. The pH of the leachates collected from amended samples was higher (2.0 < pH < 2.5) than that of the leachates from control (Fig. 1).

The stability field diagram of melanterite (Knoll et al., 2005) shows that this solid phase can be associated with minerals of the alunite-jarosite group, which are stable phases at a pH between 1.5 and 4 (Knoll et al., 2005), as well as with minerals of the copiapite-group, which only exist at a pH<1.5.

Humboldtine and moolooite can be formed by the reaction of metal ions with the oxalic acid produced by the fungi that grew in the organic amendments during the present experimental assay. Gadd (1999) analysed the biogeochemical importance of the sparingly soluble iron and copper oxalates produced by fungi.

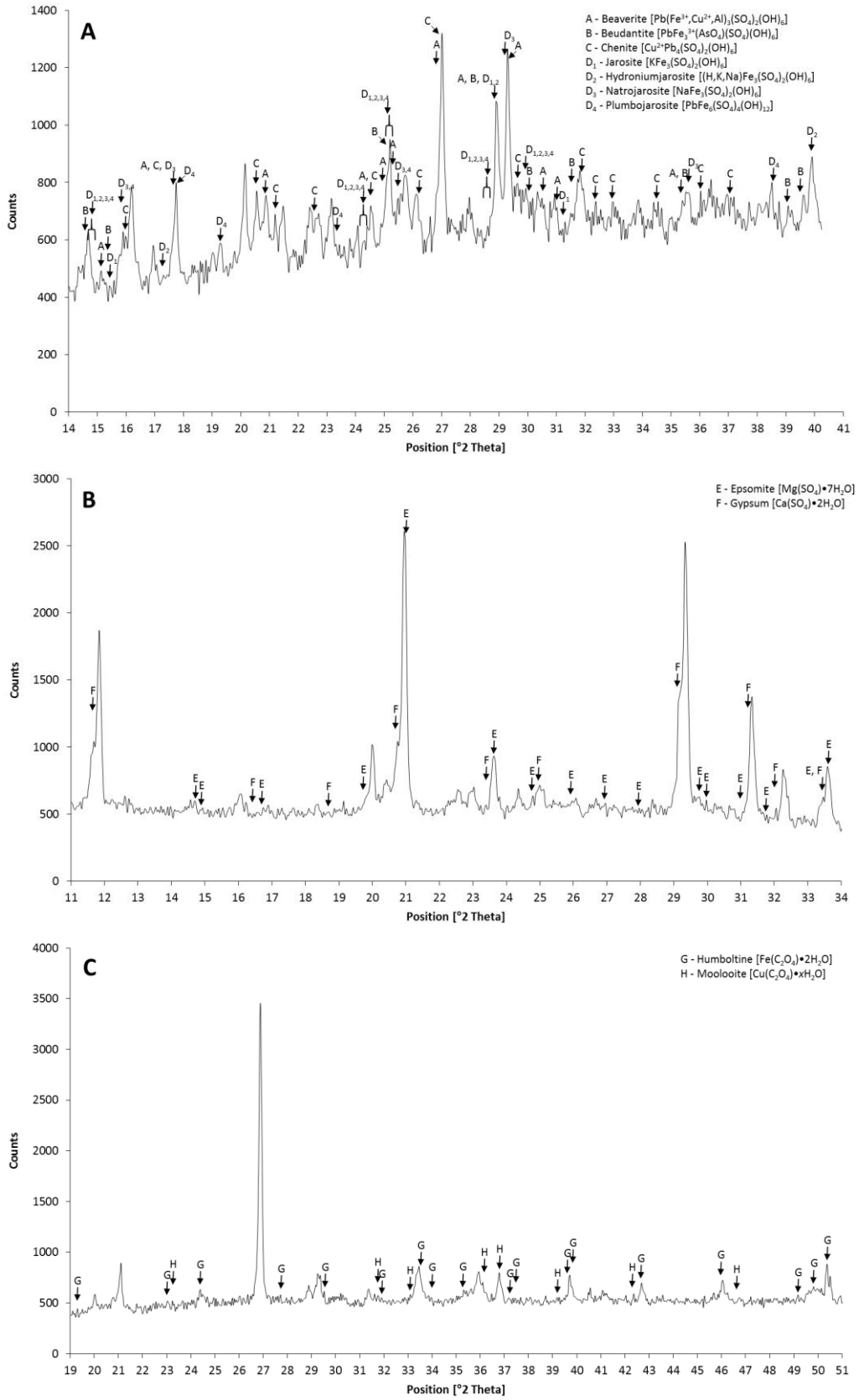

Fig. 7 X-ray diffractograms of three samples (A, B and C) of the surface efflorescent salts identified in amended materials collected after thirteen months of incubation. Peaks not identified should be attributed to organic compounds from amendments.

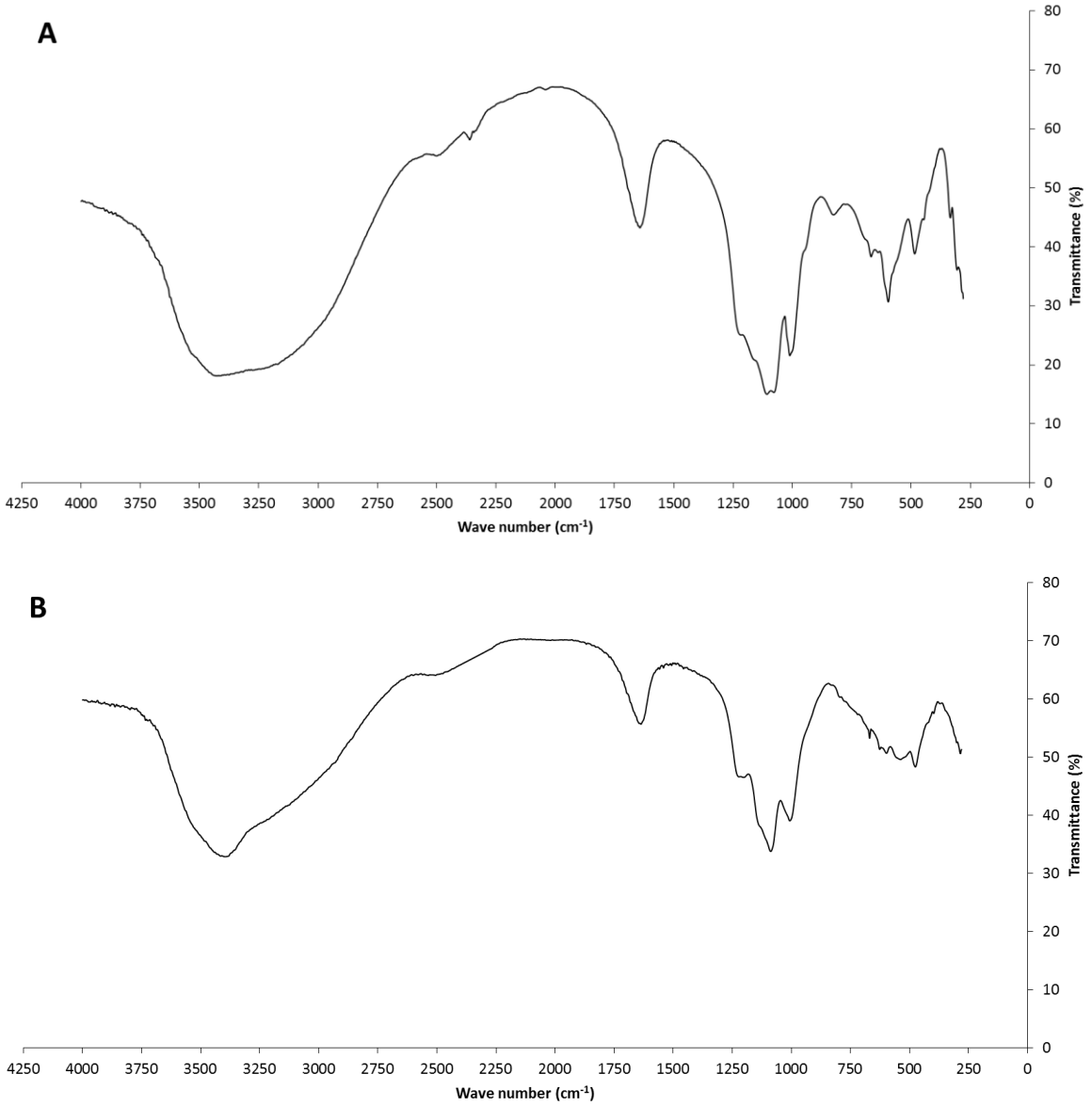

Fig. 8 Infrared spectra of surface efflorescent salts containing *copiapite*-group (A: control treatment after thirteen months of incubation) and *jarosite*-group (B: amended treatment after thirteen months of incubation).

Solid phases in the pots' core materials

In general, the same secondary solid phases were identified in materials from both depths (superficial: 0–5 cm; pot's core: ≈ 10 cm), and from control and amended samples (30 and 75 Mg/ha) (Figs. 9 and 10), which had a similar composition to that of the efflorescent salts from amended samples (Figs. 4−7). Several solid phases or solid solutions (with different degrees of metal ions substitution) belonging to the alunite-jarosite-group [beaverite, beudantite, (hydronium-, natro-, plumbo-) jarosite and segnitite ($Pb(Fe^{3+})_3H(AsO_4)_2(OH)_6$)] were the most common both in control and amended samples. Belonging to the alunite-jarosite-group, it is probable that philipsbornite ($PbAl_3H(AsO_4)_2(OH)_6$) crystallises in the control (Fig. 9), and corkite in the amended samples (Fig. 10). In addition to the abovementioned solid phases, it is highly likely that arsenbrackebuschite

($Pb_2(Fe^{2+},Zn)(AsO_4)(HAsO_4)(OH)$), berlinite ($AlPO_4$) and the minerals of the apatite-group [hedyphane ($Ca_2Pb_3(AsO_4)_3Cl$) and mimetite ($Pb_5(AsO_4)_3(OH)$] crystallised in the core of the amended samples (Fig. 10). The pH values of the waste materials (Table 3) and the leachates during the thirteen months of incubation (Fig. 1) indicate that all the core solid phases were under conditions (pH > 1.5) that promoted the crystallisation of the observed solid phases. The wastes in the pots core were kept moistened (water-holding capacity), but were not waterlogged during the time span of the experiment. This allows the creation of very localised microsystems with very specific and different environments (e.g., moist content and oxidation-reduction potential) generated by the pore system (different pore sizes), creating a diversity of chemical conditions (pH, aqueous ionic concentrations, water activity) that can produce multiple solid phases stable under each particular condition.

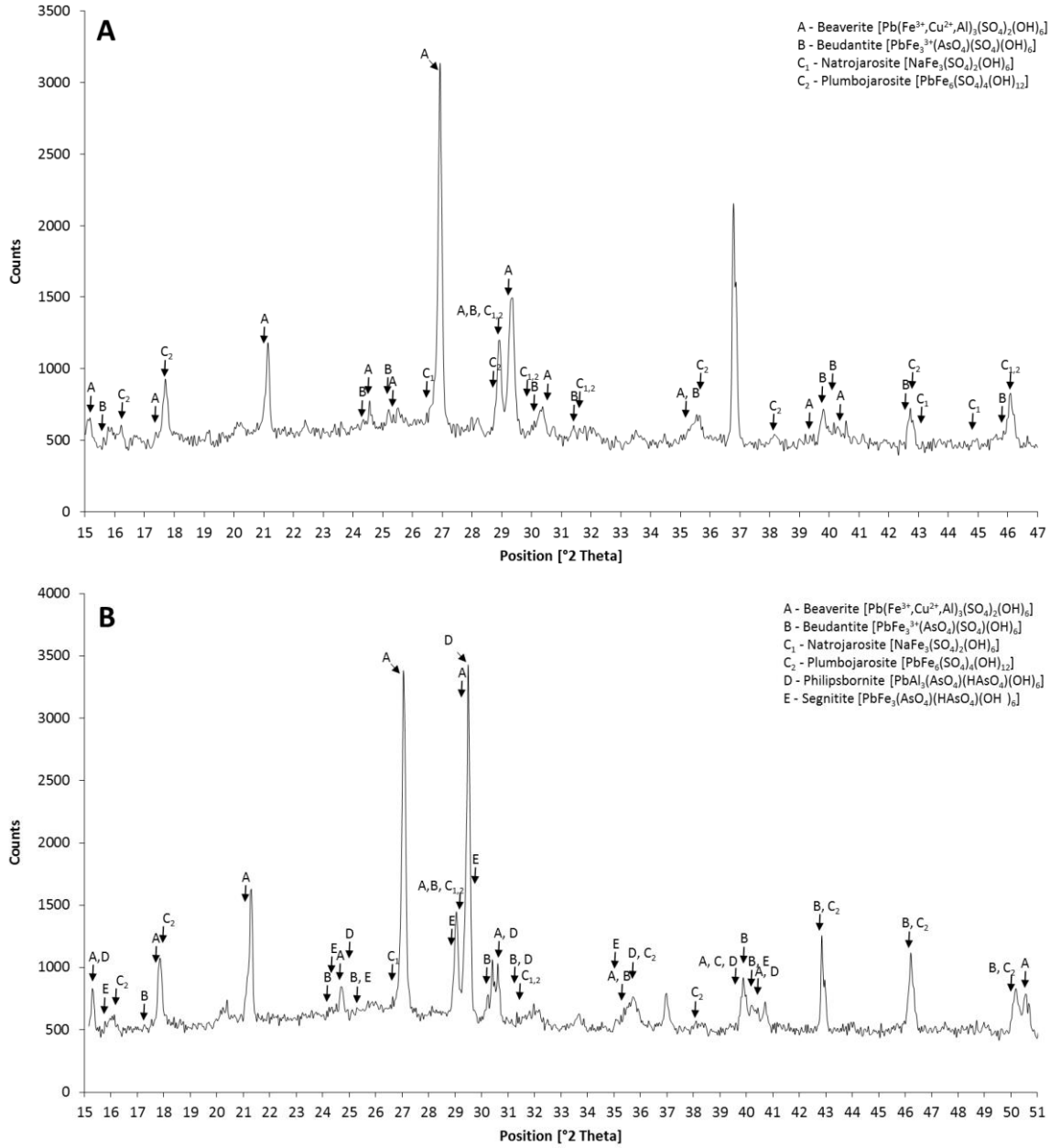

Fig. 9 X-ray diffractograms of the minerals identified in control mine waste materials, collected in two depths (A: <5 cm; B: ≈10 cm) after thirteen months of incubation.

A global analysis of the results shows that the minerals of the alunite-jarosite-group were the stable solid phases under the physical and chemical experimental environments used. The presence of these minerals, both in the core materials and as efflorescent salts on the amended wastes, means that they are moderately soluble, since the solutions that rise to the surface by capillarity can generate the same solid phases. However, the significant retention of trace elements in solid phases (Tables 4 and 5) and therefore the decrease of their concentration in leachates (Figs. 2 and 3) can be explained by the formation of these stable solid phases, under these experimental conditions.

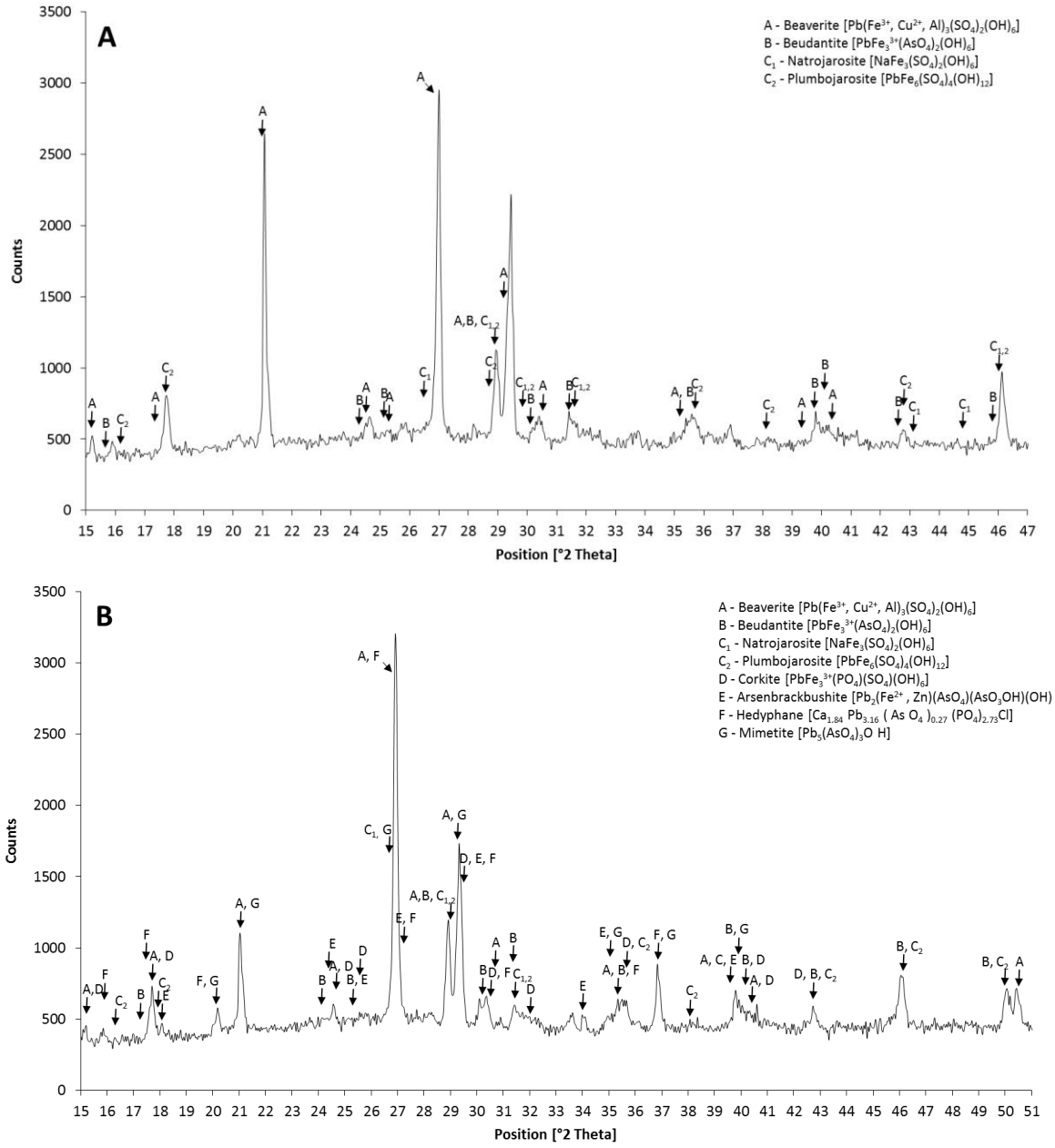

Fig. 10 X-ray diffractograms of the minerals identified in amended mine waste materials, collected in two depths (A: <5 cm; B: ≈10 cm) after thirteen months of incubation.

Table 5 Multielemental composition of minerals (µg or mg/g Dry weight) from sulfide mine wastes without (control) and with amendments application at 30 and 75 Mg/ha, collected in two depth (A: < 5 cm; B: ≈10 cm) after thirteen months of incubation.

Samples	Al (mg/g)	As (µg/g)	Ca (mg/g)	Cu (mg/g)	Fe (mg/g)	K (µg/g)	Mg (mg/g)	Mn (mg/g)	Na (µg/g)	Pb (µg/g)	Zn (mg/g)	SO_4 (mg/g)	PO_4 (µg/g)
Control – A*	0.22	26.68	4.01	0.18	4.06	2.34	0.05	0.07	42.23	18.79	0.12	58.84	48.63
Control – B*	0.32	1.53	2.82	0.06	0.87	1.32	0.02	0.004	6.54	1.44	0.05	nd	nd
Amended at 30 Mg/ha – A	0.92	5.74	4.31	0.43	3.83	2.15	0.19	0.22	< 0.60	< 2.5	0.27	34.96	27.98
Amended at 30 Mg/ha – B	0.52	3.12	8.06	0.14	1.23	1.06	0.06	0.01	4.22	0.74	0.12	nd	nd
Amended at 75 Mg/ha – A*	1.36	9.54	4.53	0.55	3.88	2.20	0.31	0.07	< 0.60	< 2.5	0.41	35.01	44.21
Amended at 75 Mg/ha – B*	0.30	2.62	8.88	0.12	0.95	1.24	0.05	0.01	4.41	0.16	0.07	nd	nd

Nd: not determined. *Samples indicated with asterisk correspond to the samples analysed by DRX.

The amount of trace elements leached in each sampling period is not enough to explain the sharp decrease in elemental concentrations in the leachate samples collected in the thirteen month.

Although iron oxides occur usually in mines after sulfide oxidation (Abreu et al., 2010; Rosado et al., 2008; Sánchez-España, 2008; Sánchez-España et al., 2005; Valente and Gomes, 2009), no iron oxides were identified due to the low pH of the waste materials. The abovementioned identified solid phases, as for instance melanterite and jarosite, show that the iron was in the structure of those solids.

CONCLUSIONS

The waste dumps containing sulfides displayed very low pH and a large capacity to leach chemical elements due to the continuous generation of acid mine drainage, and their large multielemental concentrations.

The amendments had very low pH buffer capacity in relation to the acid generated by the mine wastes. They were efficient in decreasing the elemental concentrations in leachates, compared to control, but only for a short period. However, during the thirteen months, the leached fraction of As, Fe and S from the amended sulfide materials were significantly decreased when compared to the control. The organic materials from the amendments improved the materials structure, increasing water retention and preventing excessive dryness with the appearance of local very high acidic conditions ($pH < 1$).

Low concentrations of trace elements in leachates from all treatments after thirteen months could be due to the presence, mainly in the core materials, of several solid phases with moderate solubility. Melanterite can be associated with copiapite-group minerals and also with alunite-jarosite-group minerals. Nevertheless, the higher solubility of the minerals of the copiapite-group than those of the alunite-jarosite-group means that the former solid phases crystallise from more concentrated solutions with lower pH and lower water activity. For a pH lower than 1.5, it is very likely that rozenite is a more stable solid phase than melanterite, which contains more crystallisation water molecules.

The presence of copiapite-group minerals in the efflorescent salts of control samples indicates the existence of more extreme conditions ($pH < 1.5$, high elemental concentrations and low water activity) compared to the other situations (control core materials, and efflorescent salts and core materials in amended wastes) where the alunite-jarosite-group minerals were identified. Despite the moderate solubility of the alunite-jarosite-group minerals, their crystallisation, together with arsenbrackebuschite, berlinite and the apatite-group minerals hedyphane and mimetite-(OH), can be used to explain the sharp decrease in the concentrations of the studied trace elements in the leachates collected in the thirteen month of incubation. A deep analysis of the final values attained after this time, combined with the leachates pH, illustrated that the crystallisation of these solid phases was not suitable for environmental remediation, due to the high elemental concentrations still present in the leachates. On the other hand, the stability of jarosite in the pH range between ~ 1.5 and ~ 4.5 confirms the previous conclusion that this solid phase, as well as the majority of the minerals of the alunite-jarosite-group, are not suitable for chemical elements immobilization, as they will change to different solid phases when the pH increases.

The amendments improved, in general, the chemical characteristics of the mine wastes. The dose of the amendments did not influence leachates characteristics during the entire experiment. Other organic and inorganic wastes, as well as their rate of application, should be studied over a longer time span in order to reduce AMD generation and chemical elements leaching and spread from sulfide mine wastes.

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REFERENCES

Abreu, M.M., Tavares, M.T., Batista, M.J., 2008. Potential use of *Erica andevalensis* and *Erica australis* in phytoremediation of sulphide mine environments: São Domingos, Portugal. Journal of Geochemical Exploration 96(2-3), 210-222.

Abreu, M.M., Magalhães, M.C.F., 2009. Phytostabilization of soils in mining areas. Case studies from Portugal. In: Aachen, L., Eichmann, P. (Eds.), Soil remediation. Nova Science Publishers, Inc. New York, pp. 297-344.

Abreu, M.M., Santos, E.S., Magalhães, M.C.F., Nabais, C., 2009. Arsenic containing phases in soils from São Domingos mining area and in non-contaminated soils from Pomarão and Serra do Caldeirão. Revista de Ciências Agrárias XXX (1), 155-169.

Abreu, M.M., Batista, M.J., Magalhães, M.C.F., Matos, J.X., 2010a. Acid mine drainage in the Portuguese Iberian Pyrite Belt. In: Brock C.R. (Eds.), Mine drainage and related problems. Nova Science Publishers, Inc. New York, pp. 71-118.

Activation Laboratories, 2011a. 1H - Total Digestion - ICP, INAA. Available in: www.actlabs.com/page.aspx?page=506&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes2010 (Accessed November 2011).

Activation Laboratories, 2011b. Code 6: HR-ICP/MS - Hydrogeochemistry using High Resolution ICP/MS. Available in: http://www.actlabs.com/page.aspx?page=755&app=226&cat1=549&tp=12&lk=no&menu=64

Álvarez-Valero, A.M., Pérez-López, R., Matos, J., Capitán, M.A., Nieto, J.M., Sáez, R., Delgado, J., Caraballo, M., 2008. Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian Peninsula): evidence from a chemical and mineralogical characterization. Environmental Geology 55(8), 1797-1809.

Basta, N.T., Gradwohl, R., Snethen, K.L., Schroder, J.L., 2001. Chemical immobilization of lead, zinc, and cadmium in smelter-contaminated soils using biosolids and rock phosphate. Journal of Environmental Quality 30, 1222-1230.

Cao, X., Maa, L.Q., Shiralipourb, A., 2003. Effects of compost and phosphate amendments on arsenic mobility in soils and arsenic uptake by the hyperaccumulator, *Pteris vittata* L.. Environmental Pollution 126, 157-167.

Ciccu, R., Ghiani, M., Peretti, R., Serci, A., Zucca, A., 2001. Heavy metal immobilization using fly ash in soils contaminated by mine activity. International Ash Utilization Symposium. Available in: www.flyash.info/2001/mining1/06perett.pdf. (Accessed November 2011).

Fitz, W.J., Wenzel, W.W., 2002. Arsenic transformations in the soil rhizosphere-plant system: fundamentals and potential application to phytoremediation. Journal of Biotechnology 99(3), 259-278.

Forsberg, L.S., Gustafsson, J., Kleja, D.B., Ledin, S., 2008. Leaching of metals from oxidising sulphide mine tailings with and without sewage sludge application. Water, Air and Soil Pollution 194, 331-341.

Gadd, G.M., 1999. Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical processes. Advances in Microbial Physiology 41, 47-92.

Gil Bueno, A., Val Caballero, C., Macías, F., Monterroso, C., 1990. Influence of waste selection in the dump reclamation at Puentes Mine. In: Rainbow (Ed.), Reclamation, treatment an utilization of coal mining wastes. Bolkema, Rotterdam, pp. 203-208.

Jamieson, H.E., Robinson, C., Alpers, C.N., McCleskey, R.B., Nordstrom, D.K., Peterson, R.C., 2005a. Major and trace element composition of copiapite-group minerals and coexisting water from the Richmond mine, Iron Mountain, California. Chemical Geology 215, 387-405.

Jamieson, H.E., Robinson, C., Alpers, C.N., Nordstrom, D.K., Poustovetov, A., Lowers, H.A., 2005b. The composition of coexisting jarosite-group minerals and water from the Richmond mine, Iron Mountain, California. The Canadian Mineralogist 43, 1225-1242.

Kabata-Pendias, A., Pendias, H., 2001.Trace elements in soils and plants. CRC Press, Boca Raton.

Knoll, A.H., Carr, M., Clark, B., Des Marais, D.J., Farmer, J.D., Fischer, W.W., Grotzinger, J.P., Hayes, A., McLennan, S.M., Malin, M., Schröder, C., Squyres, S., Tosca, N.J., Wdowiak, T., 2005. An astrobiological perspective on Meridiani Planum. Earth and Planetary Science Letters 240, 179-189.

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments − a review. Waste Management 28, 215-225.

Macías, F., 2004. Recuperación de suelos degradados, reutilización de resíduos y secuestro de carbono. Una alternativa integral de mejora de la calidad ambiental. Recursos Rurais 1, 49-56.

Matos, J.X., 2004. Carta geológica e mineira da Mina de S. Domingos esc. 1/5000, IGM.

Matos, J.X., Martins, L.P., 2006. Reabilitação ambiental de áreas mineiras do sector português da Faixa Piritosa Ibérica: estado da arte e perspectivas futuras. Boletín Geológico y Minero 117, 289-304.

McBride, M.B., 2003. Toxic metals in sewage sludge-amended soils: has promotion of beneficial use discounted the risks?. Advances in Environmental Research 8, 5-19.

Mindat - Mindat.org - the mineral and locality database, 2013. Available in: http://www.mindat.org/

Moenke, H., 1962. Mineralsperktren. Akademie Verlag, Berlin.

Nieto, J.M., Sarmiento, A.M., Olías, M., Canovas, C.R., Riba, I., Kalman, J.T., Delvalls, A., 2007. Acid mine drainage pollution in the Tinto and Odiel rivers (Iberian Pyrite Belt, SW Spain) and bioavailability of the transported metals to the Huelva Estuary. Environment International 33, 445-455.

Pérez-López, R., Nieto, J.M., Almodóvar, G.R., 2007. Immobilization of toxic elements in mine residues derived from mining activities in the Iberian Pyrite Belt (SW Spain): Laboratory experiments. Applied Geochemistry 22, 1919-1935.

Povoas, I., Barral, M.F., 1992. Métodos de análise de solos. Comunicações do Instituto de Investigação Científica Tropical. Serie Ciências Agrárias 10, Lisboa.

Quental, L., Bourguignon, A., Sousa, A.J., Batista, M.J., Brito, M.G., Tavares, T., Abreu, M.M.,Vairinho, M., Cottard, F., 2002. MINEO Southern Europe environment test site. Contamination impact mapping and modelling - Final Report. Assessing and monitoring the environmental impact of mining activities in Europe using advanced Earth Observation Techniques (MINEO) 5yth FP-IST-1999-10337.

Rodríguez-Jordá, M.P., Garrido, F., García-González, M.T., 2010. Potential use of gypsum and lime rich industrial by-products for induced reduction of Pb, Zn and Ni leachability in an acid soil. Journal of Hazardous Materials 175, 762-769.

Rodríguez-Jordá, M.P., Garrido, F., García-González, M.T., 2012. Effect of the addition of industrial by-products on Cu, Zn, Pb and As leachability in a mine sediment. Journal of Hazardous Materials 213-214, 46-54.

Romero, A., González, I., Galán, E., 2006. The role of efflorescent sulfates in the storage of trace elements in stream waters polluted by acid mine-drainage: the case of Peña del Hierro, Southwestern Spain. The Canadian Mineralogist 44, 1431-1446.

Rosado, L., Morais, C., Candeias, A.E., Pinto, A.P., Guimarães, F., Mirão, J., 2008. Weathering of S. Domingos (Iberian Pyritic Belt) abandoned mine slags. Mineralogical Magazine 72(1), 489-494.

Sáinz, A., Grande, J.A., De la Torre, M.L., 2003. Analysis of the impact of local corrective measures on the input of contaminants from the Odiel River to the Ría of Huelva (Spain). Water, Air and Soil Pollution 144, 375-389.

Sánchez-España, J., 2008. Acid mine drainage in the Iberian Pyrite Belt: an overview with special emphasis on generation mechanisms, aqueous composition and associated mineral phases. Macla 10, 34-43.

Sánchez-España, J., Pamo, E.L., Santofimia, E., Aduvire, O., Reyes, J., Barettino, D. 2005. Acid mine drainage in the Iberian Pyrite Belt (Odiel river watershed, Huelva, SW Spain): Geochemistry, mineralogy and environmental implications. Applied Geochemistry 20, 1320-1356.

Sánchez-España, J., Toril, G.E., Pamo, E.L., Amils, R., Ercilla, M.D., Pastor, E.S., San Martín-Úriz, P., 2008. Biogeochemistry of hyperacid and ultraconcentrated pyrite leachate in San Telmo mine (Iberian Pyrte Belt, Spain). Water, Air and Soil Pollution 194, 243-257.

Santos, E.S., Abreu, M.M., Nabais, C., Magalhães, M.C.F., 2012. Trace elements distribution in soils developed on *gossan* mine wastes and *Cistus ladanifer* L. tolerance and bioaccumulation. Journal of Geochemical Exploration 123, 45-51.

Sneddon, I.R., Orueetxebarria, M., Hodson, M.E., Schofield, P.F., Valsami-Jones, E., 2006. Use of bone meal amendments to immobilise Pb, Zn and Cd in soil: A leaching column study. Environmental Pollution 144, 816-825.

Sullivan, P.J., Yelton, J.L., 1988. An evaluation of trace element release associated with acid mine drainage. Environmental Geology and Water Sciences 12, 181-186.

USEPA – United States Environmental Protection Agency, 1979. Methods for chemical analysis of water and waste. Office of Research and Development, US Environmental Protection Agency, method 365.2, Cincinatti, Ohio.

Valente, T.M., Gomes, C.L., 2009. Occurrence, properties and pollution potential of environmental minerals in acid mine drainage. Science of Total Environment 407(3), 1135-1152.

Zanuzzi, A., Arocena, J.M., van Mourik, J.M., Cano, A.F., 2009. Amendments with organic and industrial wastes stimulate soil formation in mine tailings as revealed by micromorphology. Geoderma 154, 69-75.

8. CHEMICAL QUALITY OF LEACHATES AND ENZIMATIC ACTIVITIES IN TECHNOSOLS WITH GOSSAN AND SULFIDE WASTES FROM THE SÃO DOMINGOS MINE

ABSTRACT

São Domingos mine area (Portuguese Iberian Pyrite Belt) contains several mine wastes with different environmental hazard which are disposed irregularly over a large area. This study aimed to evaluate: i) soil enzymatic activities, chemical quality of leachates and *Cistus ladanifer* germination in Technosols composed of *gossan* or sulfide wastes from the mine and amendment mixtures (organic and inorganic); ii) the efficiency of a layer of *gossan* over sulfide wastes to improve rehabilitation of the more hazardous waste.

Technosols were composed of *gossan* or sulfide wastes and amendment mixtures, at 12 and 30 g/kg, containing organic/inorganic wastes from agriculture and distilleries in equal proportion. Three microcosm assays, under controlled conditions, were carried out: *Gossan*/sulfide wastes and respective Technosols (assays 1-2); (assay 3) Sulfide wastes, with/without amendments, incubated during four months and then with an application of a thin overlayer of *gossan* with/without the same amendments. *Cistus ladanifer* was sown in half of the pots from each treatment while the remainder was left bare. After three and nine months of incubation, composite samples of the materials were collected and dehydrogenase, acid phosphatase and β-glucosidase activities were determined. In same sampling periods, percolation leachates were analysed for anions and cations concentrations, pH and electrical conductivity.

Technosols with *gossan* wastes were significantly different ($p < 0.05$) due to higher dehydrogenase and β-glucosidase activities, better seedling growth and improved chemical characteristics of leachates. Although electrical conductivity and concentrations of some elements (*e.g.* As and SO_4) in leachates from Technosols with sulfide wastes were reduced (>50 % of Electrical conductivity and 23–99 % depending on element, Technosol and sampling period), the amendments did not prevent sulfide oxidation and acid generation, improve enzymatic activities or allow seed germination. Higher elements concentrations in leachates were obtained with the overlapping of two Technosols or mine wastes, compared to the leachates from assays with a single mine waste. Application of a *gossan*/Technosol layer over sulfide wastes allowed C. ladanifer germination but plants died after ≈50 days.

The use of Technosols derived from *gossan* or sulfide wastes may be an efficient solution for rehabilitation of these mine wastes. However, the results with sulfide wastes were not encouraging. The use of a Technosol obtained from *gossan* wastes seems be a promising approach to isolate sulfide wastes from air and initiate their revegetation, but this technology requires further improvements, namely to prevent the ascension of salts by capillarity.

KEYWORDS *Cistus ladanifer* germination • Multielementar contamination • Organic/inorganic wastes • Vegetation support

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INTRODUCTION

Different wastes were produced by mining activities in São Domingos (Portuguese Iberian Pyrite Belt) and disposed over a large area affecting around 3.2 x 10^6 m^2 of land (Álvarez-Valero et al., 2008; Matos and Martins, 2006; Quental et al., 2002). Each mine waste poses different risks associated to its chemical composition and total concentrations of hazardous elements (Álvarez-Valero et al., 2008). Although this evaluation is important, real environmental impacts of mine wastes are related to the available fraction of the elements and mass/volume of the heaps (Pérez-López et al., 2008). This is because only elements in aqueous solution or in easily exchangeable positions in soils or wastes are directly available to soil organisms (Adriano, 2001). Taken into account the mobility of trace elements and the mass/volume of the heaps, *gossan* and brittle pyrite wastes represent the fourth and fifth, respectively, most hazardous mine wastes in the São Domingos mine area (Pérez-López et al., 2008).

To speed up ecological rehabilitation of these wastes, a combined strategy using different organic and inorganic amendments and mine wastes can be used to create specific Technosols (Macías, 2004; Macías et al., 2011). The mail goal of these Technosols should be to improve the characteristics of mine wastes: chemical (*e.g.* increase in nutrients and organic matter and reduction of acid drainage and availability of hazardous elements), physical (*e.g.* substrata structure) and biological (enhanced plant cover and activity of soil microorganisms to promote elements cycling processes). Moreover, the development of a plant cover, namely of *Cistus ladanifer* L., can stimulate ecosystem functions of Technosols containing *gossan* wastes (Santos et al., 2014a).Cistus ladanifer is a pioneer species that grows spontaneously in several mine areas in the Iberian Pyrite Belt due to it efficient mechanisms of tolerance (Alvarenga et al., 2004; Batista et al., 2007; Freitas et al., 2004; Santos et al., 2009, 2012, 2013a, 2014b).

In general, soil quality depends on several physical, chemical, biological and biochemical properties (Kumar et al., 2013). Soil enzyme activities are very sensitive with a fast response to induced changes during degradation/contamination and ecological rehabilitation (Karlen et al., 1997; Santos et al., 2013b, 2014a; de Varennes et al., 2010a). Determination of different soil enzymatic activities can also indicate potential variation in microbial diversity. However, few studies are available on the dynamics of enzymatic activities in mine wastes or soils developed on mine wastes, with/without amendments, for long periods of monitoring (Burgos et al., 2010; Santos et al., 2014a).

This study aimed to evaluate: i) soil enzymatic activities, chemical quality of leachates and *Cistus ladanifer* germination in Technosols composed of *gossan* or sulfide wastes from São Domigos mine and amendment mixtures (organic and inorganic); ii) the efficiency of a layer of *gossan* over sulfide wastes (with/without amendments) to improve rehabilitation of the more hazardous waste. We hypothesized that the conception of Technosols with amendments mixtures and mine wastes would increase enzymatic activities related to biogeochemical cycles and improve the chemical quality of leachates in order to decrease the dispersion of elements and allow the development of pioneer vegetation. Moreover, the overlapping of Technosolos would isolate sulfide wastes from air/water what would promote the plant cover and, consequently, reduce leaching of chemical elements.

MATERIALS AND METHODS

Experiment set-up

Composite samples of two mining wastes, which are composed of *gossan* materials mixed with host rocks (hereinafter named as *gossan* wastes − GW) and crushed pyrite and smelting ashes (herein after named as sulfide wastes − SW), and Cistus ladanifer seeds were collected in the São Domingos mine area in 2009. Sampling area for GW was located near the open pit and for SW was south-west of the open pit, near the former leaching tanks. These mine wastes were chosen due to the large amounts of trace elements present and consequent potential environmental risks, wide distribution in the mine area (Álvarez-Valero et al., 2008; Pérez-López et al., 2008; Quental et al., 2002) and level of plant colonization. The SW are bare and exposed to severe water erosion while GW are naturally colonized by autochthonous plants. The chemical characteristics of the mine wastes are given in Table 1.

The Technosols (Table 2) were composed of GW or SW and an amendment mixture containing organic/inorganic wastes (equal proportion of each residue) from agriculture (plant remains + strawberry substrate at 2:3 m/m – AgW; and rockwool used for strawberry crops – RW) and residues from distilleries (*Arbutus unedo* L. and *Ceratonia siliqua* L. fruit spirits distillation – AW, CW, respectively). Three amendment mixtures were tested (AgW + AW + RW; AgW + CW + RW; and AgW + AW +CW + RW) at two application rates (12 and 30 g/kg of mine waste). Residues from a quarry (limestone rock wastes – LRW) were only added, at 22 g/kg, to SW to increase pH to similar values as that of GW. These organic and inorganic wastes were used due to their physical and chemical characteristics (Table 1) and because they are easily available in the vicinity of the mine at a very low cost.

The mine wastes and amendments were air-dried, mixed manually and put in pots. Three microcosm assays were carried out: (Assay 1) GW (fraction < 10 mm) with/without amendments (seven treatments: one control and six Technosols) with three replicates each, 2 kg of GW per pot; (Assay 2) SW (total fraction) with/without amendments (seven treatments: one control and six Technosols) with four replicates each, 2 kg of SW per pot; (Assay 3) Sulfide wastes, with/without amendments, incubated during four months and then with application of an overlayer of *gossan* (≈4 cm thick) with/without the same amendments (seven treatments: one control and six Technosols) with four replicates each, 2 kg of GW and 4 kg of SW per pot. To evaluate the efficiency of the application of an overlayer of *gossan*, GW or SW with amendments (Technosols) were used as positive controls while GW or SW acted as negative controls.

Cistus ladanifer was sown (0.5 g seeds per pot) in half of the pots from each treatment, while the other pots remained bare. The seeds were pre-treated for optimal germination (heating at 100 °C for 30 min; Corral et al., 1990). All treatments were kept at 70 % of the maximum water-holding capacity under controlled conditions in a greenhouse and monitored for nine months (July-April).

Table 1 Chemical characteristics of the *gossan* and the sulfide wastes from São Domingos mine area and organic/inorganic wastes used as amendments (min – max or mean value) (Santos et al. 2014a,b).

	GW	SW	AgW	AW	CW	RW
pH (H_2O)	4.32	2.1 – 2.3	6.6 – 7.2	4.9	6.1	7.1
Electrical conductivity (mS/cm)	<0.1	7.4 – 7.5	0.9 – 3.5	1.7	0.2	3.5
Organic C (g/kg)	12.0	243.8 – 276.1*	286.1	442.6	436.7	111.0
Total N (g/kg)	0.04	3.7 – 4.8	9.7	8.9	11.1	9.8
Extractable P (g/kg)	1.3×10^{-3}	< DL	0.3 – 3.1	0.2	0.1	7.9
Extractable K (g/kg)	0.08	0.02 – 0.06	0.2 – 6.6	3.6	9.1	0.7
	Total concentrations (g/kg dry weight)					
Al	24.8	54.8 – 61.2	4.06	1.37	0.6×10^{-3}	53.4
As	3.03	1.02 – 1.14	2.3×10^{-3}	1.0×10^{-3}	0.8×10^{-3}	$< 0.5 \times 10^{-3}$
Ca	0.5	6.40 – 6.70	22.56	10.30	11.02	139.0
Cu	0.23	1.84 – 2.45	44.6×10^{-3}	0.12	9.4×10^{-3}	0.08
Fe	129	104 – 110	2.96	3.30	0.99	39.90
K	21.2	12.8 – 14.3	1.495	> 1.32	0.96	6.80
Mg	1.2	1.4 – 1.8	2.34	1.13	> 9.63	42.10
Mn	62.0×10^{-3}	0.12 – 0.16	0.25	51.2×10^{-3}	43.1×10^{-3}	1.85
Na	1.7	4.20 – 4.60	0.31	0.40	0.40	13.10
Pb	1.08	8.97 – 13.80	5.0×10^{-3}	3.1×10^{-3}	0.9×10^{-3}	$< 3 \times 10^{-3}$
S	9.21	63.6 – 68.0	NA	NA	NA	2.60
Zn	13.7	1.08 – 1.19	75.6×10^{-3}	27.3×10^{-3}	18.5×10^{-3}	0.20

*Total C concentration; GW: *gossan* wastes; SW: sulfide wastes; AgW: Agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops; DL: detection limit; NA: non analysed

Table 2 Composition of the Technosols used in the three assays.

Mine waste	Amendment mixture	Rate of application (g/kg of mine waste) (designation)	Other inorganic wastes	Rate of application (g/kg of mine waste)
Gossan wastes (GW) Assay 1	AgW + AW + RW	12 (GA12), 30 (GA30)	---	---
	AgW + CW + RW	12 (GC12); 30 (GC30)	---	---
	AgW + AW + CW + RW	12 (GAC12); 30 (GAC30)	---	---
Sulfide wastes (SW) Assay 2	AgW + AW + RW	12 (SA12), 30 (SA30)	LRW	22
	AgW + CW + RW	12 (SC12); 30 (SC30)	LRW	22
	AgW + AW + CW + RW	12 (SAC12); 30 (SAC30)	LRW	22
Gossan and sulfide wastes (GW/SW) (independent layers) Assay 3	AgW + AW + RW	12 ((G/S)A12), 30 ((G/S)A30)	LRW only in SW	22
	AgW + CW + RW	12 ((G/S)C12); 30 ((G/S)C30)	LRW only in SW	22
	AgW + AW + CW + RW	12 ((G/S)AC12); 30 ((G/S)AC30)	LRW only in SW	22

AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops; LRW: limestone rock wastes

Experimental monitoring and sample analyses

At the beginning and after three and nine months (end of the experiment), composite samples of the materials from each pot were collected (assays 1 and 2: < 3 cm of depth; assay 3: < 3 cm (*gossan* materials) and 5−7 cm of depth (sulfide materials). These materials were homogenised, sieved (< 2 mm) and kept fresh (4 ºC) until soil enzymatic analysis.

Three enzymatic activities were analysed as biological parameters to evaluate the rehabilitation process: dehydrogenase (Tabatabai, 1994), β-glucosidase (EC 3.2.1.21; Eivazi and Tabatabai, 1988) and acid phosphomonoesterase (acid phosphatase EC 3.1.3.2; Eivazi and Tabatabai, 1977). Dehydrogenase was used as an indicator of overall microbial activity while other enzymatic activities are related to C and P cycling.

In the same sampling periods, percolation leachates were obtained by irrigation of the materials (without plants) with a volume of deionised water that ensured an excess of 10 % of each pot's maximum water holding capacity, and left to percolate for 24 h. The leachates were vacuum filtrated (< 0.45 µm), and pH and electrical conductivity (EC) measured. The filtrate was kept at –18 ºC until analysis. In the leachates, the concentrations of Al, Ca, Cu, Fe, K, Mg, Mn, Na, Pb and Zn were analysed by flame atomic absorption spectrometry; As by graphite furnace atomic absorption spectrometry; chlorides, nitrates and sulfates by ion chromatography; and phosphates by visible spectrophotometry using the molybdenum blue method (USEPA, 1979).

Data analysis

Data were analysed by one way ANOVA and the Duncan test ($p < 0.05$) used to separate means, using the statistical programme SPSS v18.0 for Windows. Data not satisfying assumptions for ANOVA were analysed non-parametrically using Kruskal-Wallis ANOVA by a rank test. For statistical purposes, the results below the detection limit were assumed as half of the detection limit. Bivariate Pearson correlations were used to correlate leachates characteristics and soil enzymatic activities (strong correlation: $r > 0.95$; moderate correlation: $0.65 < r < 0.95$). Quality control of the analyses was made by analytical replicate samples, use of certified standards solutions and laboratory standards at an international accredited laboratory (multielemental concentration of mine and organic/inorganic wastes).

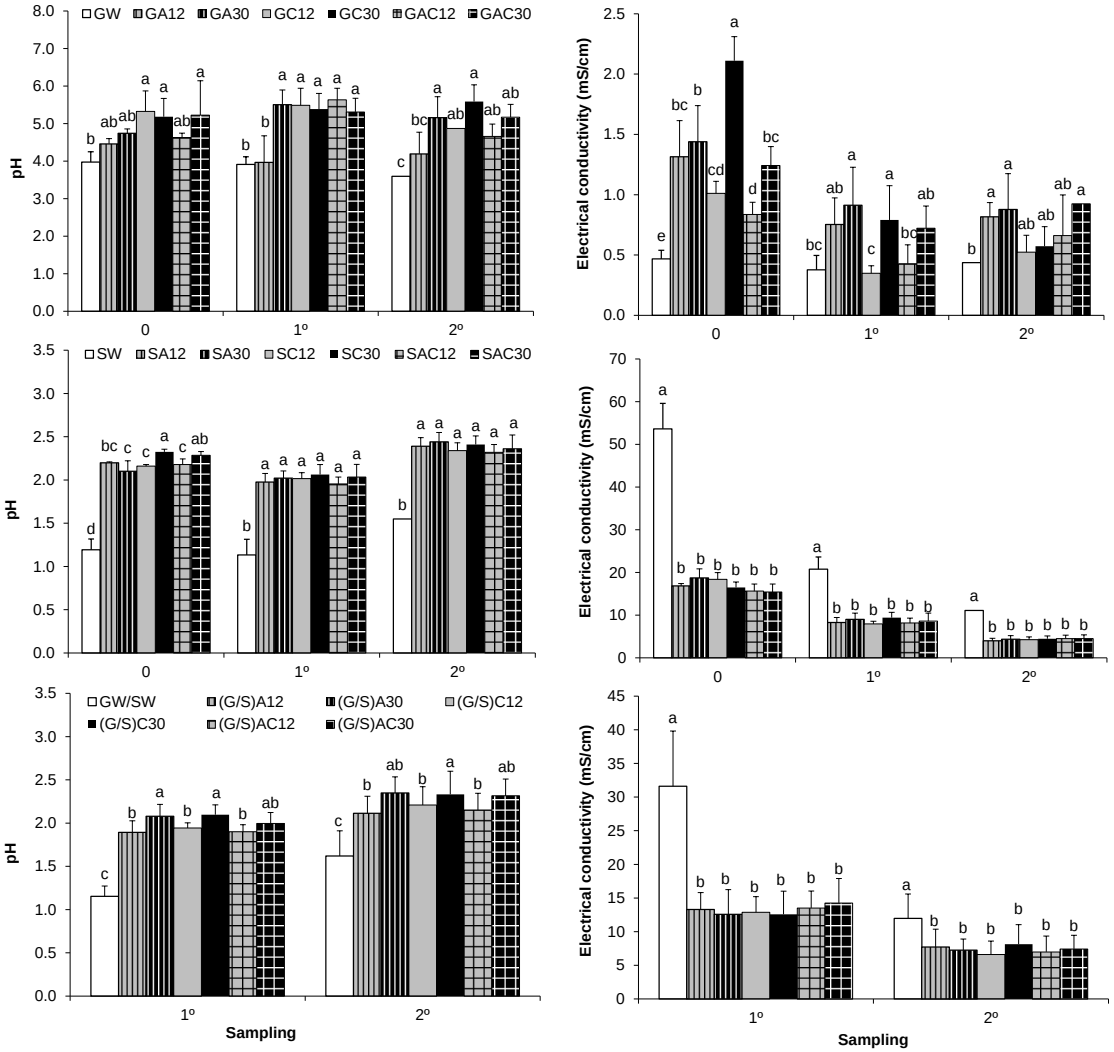
RESULTS AND DISCUSSION

Gossan wastes

Chemical characterization of leachates

The amendments increased pH of GW leachates by more than one unit (4.5–5.6, except for Technosols containing A. unedo fruit spirit distillation (GA12 and GA30) and both fruits spirits distillation and amendment mixture applied at 12 g/kg (GAC12) where pH was close to 4.5 in the first

sampling) compared to the control (3.6 < GW < 4.0; Fig. 1), with no significant differences between the three sampling dates. Similar results of pH were obtained in leachates (simulated and percolated) from contaminated soils and sediments amended with other organic/inorganic wastes (Hodson et al., 2001; Rodríguez-Jordá et al., 2012) as well as soils developed on *gossan* wastes amended with hydrophilic polyacrylate polymers (Santos et al., 2013b).



GW: *gossan* wastes; SW: sulfide wastes; GW/SW: *gossan* waste layer over sulfide waste; GA12, GA30, SA12 and SA30: Technosol containing GW or SW and AgW+AW+RW at 12 and 30 g/kg; GC12, GC30, SC12 and SC30: Technosol containing GW or SW and AgW+CW+RW at 12 and 30 g/kg; GAC12, GAC30, SAC12 and SAC30: Technosol containing GW or SW and AgW+AW+CW+RW at 12 and 30 g/kg; (G/S)A12 and (G/S)A30: Overlapping of Technosols containing GW or SW and AgW+AW+RW at 12 and 30 g/kg; (G/S)C12 and (G/S)C30: Overlapping of Technosols containing GW or SW and AgW+CW+RW at 12 and 30 g/kg; (G/S)AC12 and (G/S)AC30: Overlapping of Technosols containing GW or SW and AgW+AW+CW+RW at 12 and 30 g/kg. AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops.

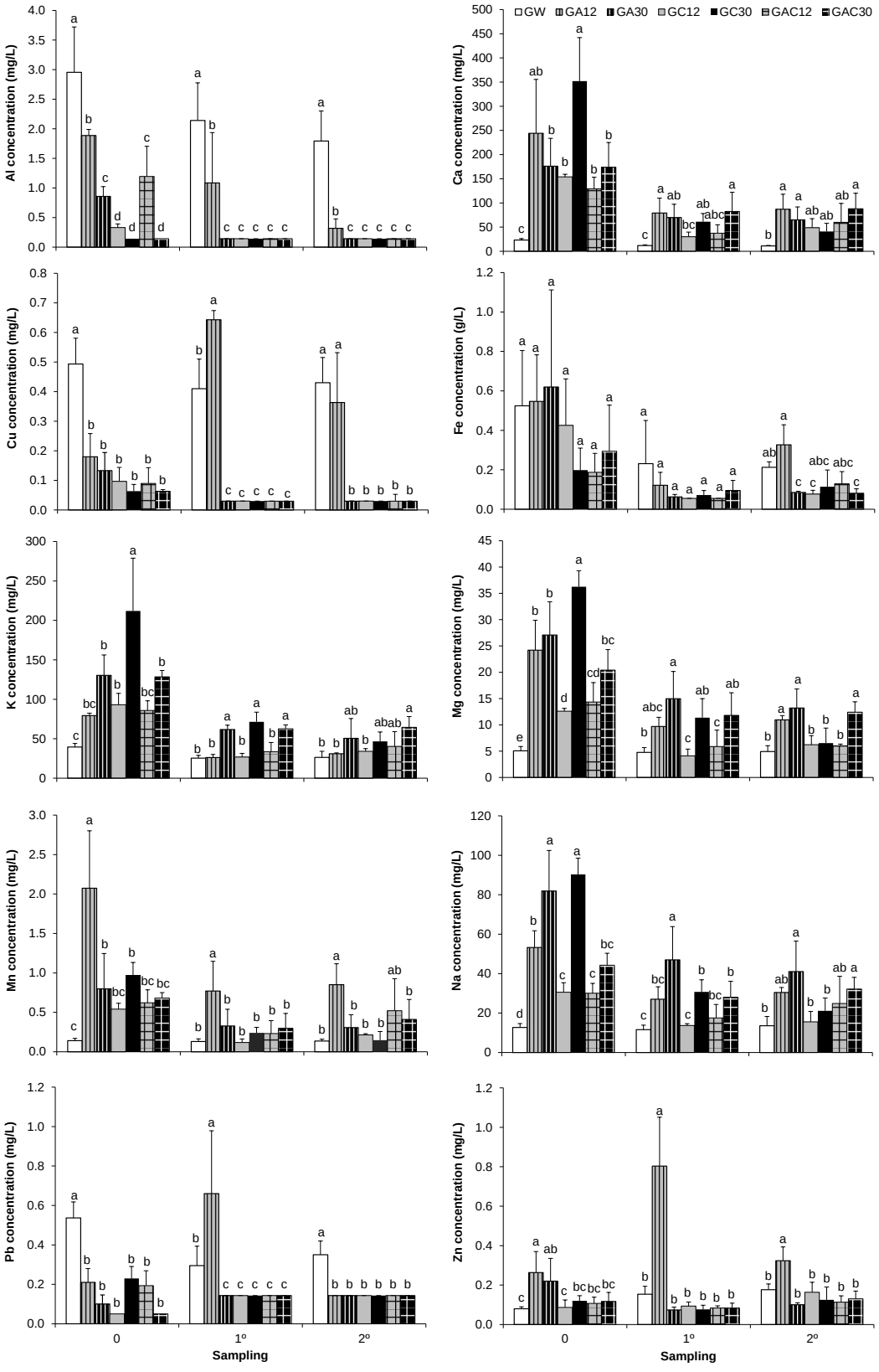
Fig. 1 pH and electrical conductivity (EC) in leachates from Technosols with *gossan* and/or sulfide wastes (Mean ± SD; *n* = 3 or 4 depending on assay). Values from same assay and sampling period followed by a different letter are significantly different ($p < 0.05$).

In the beginning of the experiment, leachates from all Technosols had electrical conductivities significantly higher than control (Fig. 1), but later on this only occurred in leachates from Technosols receiving a high dose of amendment (mS/cm; 1st sampling – GW: 0.4, GA30: 0.9, GC30: 0.8; 2nd sampling – GW: 0.4, GA12: 0.8, GA30 and GAC30: ≈0.9). Nevertheless, these values can be

considered as small.Although GW had large total concentrations of some elements (e.g. Al, As, Cu and Pb, Table 1), their concentrations in leachates, and thus potentially available for soil organisms (namely those involved in biogeochemical cycles), were small in GW with or without amendment application representing a low potential environmental risk (Table 3 and Fig. 2). Significant decreases ($p < 0.05$) in the concentrations of elements (except As) in leachates occurred in the two sampling dates. In leachates from contaminated sediments from São Domingos amended with sugar foam, ashes from combustion of biomass and red gypsum, Rodríguez-Jordá et al. (2012) also reported a two-phase time-dependent behaviour for the concentrations of Cu, Pb and Zn, with an initial decrease (until ≈20 days) and then a steady state. For As, a progressive increase in the concentration occurred during the experiment, independently of the treatment (Table 3).

In Technosols, especially those containing an amendment mixture at 12g/kg and containing A. unedo fruit spirit distillation (GA12) and different amendment mixtures at 30 g/kg (GA30, GC30 and GAC30), there was a significant increase in the concentrations of the elements in leachates (between 1.5 and 11-fold depending on the element), compared to control (Table 3 and Fig. 2). However, no clear tendency was verified according to the type of amendment, element and sampling period. Increases in the concentrations of As, Na and phosphate in simulated leachates was also reported in soils developed on *gossan* wastes but amended with hydrophilic polyacrylate polymers (Santos et al., 2013b).Depending on characteristics of amendments and mine wastes, the concentrations of elements in leachates can vary with time due to different chemical and mineralogical reactions. In Technosols, the high concentrations of chlorides and nutrients were related to nutrient solution used in strawberry cultivation, which was incorporated in rockwool (Table 1). This increase in nutrients can be considered an advantage for the phytostabilization process, namely with C. ladanifer, as reported by Santos et al. (2014a).

Comparing the elements in control and Technosols, two groups of elements that constituted exceptions were observed: As and Fe, and Al, Cu, Pb and nitrates (Table 3 and Fig. 2). In the first sampling, similar concentrations of As and Fe among leachates were obtained, usually lower than 25 µg As/L and 0.62 mg Fe/L. However, at the beginning and at the end of the experiment, As concentrations in leachates from Technosols with rate application of 30 g/kg and containing residue from liquor distillation of C. siliqua fruit (GC30: 1.40 and 76.6 µg As/L, respectively) and both residues from liquors distillation (GAC30: 7.59 and 51.8 µg As/L, respectively) were significantly higher than control (0.73 and 9.3 µg As/L, respectively). The type of organic matter derived from residue of liquor distillation of C. siliqua fruit and its content in these Technosols can play an important role in As mobility (Kumpiene et al., 2008). Moreover, the increase of low-molecular-weight organic acids following amendment application can release As from Fe–, Mn– and Al–oxides or hydroxides in contaminated soils (Zang et al., 2005). In fact, soils developed on mine wastes from São Domingos mine area can present a considerable fraction of As associated to Fe–oxides and organic matter (6–27 % and 1–34 % of total As concentrations, respectively) (Abreu et al., 2012; Santos et al., 2012). The inhibition of the formation of metal arsenates, that naturally occur in *gossan* wastes (Santos et al., 2012), may be another factor explaining the As increase in leachates.



GW: *gossan* wastes; GA12 and GA30: Technosol containing GW and AgW+AW+RW at 12 and 30 g/kg; GC12 and GC30: Technosol containing GW and AgW+CW+RW at 12 and 30 g/kg; GAC12 and GAC30: Technosol containing GW and AgW+AW+CW+RW at 12 and 30 g/kg. AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops.

Fig. 2 Concentrations of cations in leachates from Technosols and *gossan* wastes – assay 1 (Mean ± SD; $n = 3$). Values from same sampling period followed by a different letter are significantly different ($p < 0.05$).

Table 3 Concentrations of anions in leachates from Technosols and *gossan* wastes – assay 1 (Mean ± SD; *n* = 3) collected at time zero (0) and after three (1º) and nine (2º) months of incubation.

Anion	Sampling	Assay 1						
		GW	GA12	GA30	GC12	GC30	GAC12	GAC30
As (µg/L)	0	0.7 ± 0.1^{bcd}	1.3 ± 0.7^{bc}	0.7 ± 0.6^{bcd}	1.2 ± 0.2^{bc}	1.4 ± 0.1^{b}	<0.3^{cd}	7.6 ± 0.2^{a}
	1º	8.9 ± 5.7^{ab}	5.7 ± 3.6^{b}	17.1 ± 6.0^{a}	14.9 ± 0.4^{a}	12.1 ± 5.2^{a}	8.1 ± 3.3^{ab}	6.9 ± 5.6^{ab}
	2º	9.3 ± 1.7^{b}	21.0 ± 9.3^{b}	20.9 ± 9.3^{b}	25.0 ± 7.2^{b}	76.6 ± 8.9^{a}	11.0 ± 3.8^{b}	51.8 ± 24.5^{a}
Cl^- (mg/L)	0	44.4 ± 6.4^{c}	120.0 ± 13.2^{b}	196.1 ± 38.2^{a}	95.2 ± 29.9^{b}	230.7 ± 20.1^{a}	79.2 ± 18.4^{b}	110.6 ± 4.4^{b}
	1º	39.1 ± 8.6^{bc}	56.0 ± 6.5^{bc}	108.3 ± 40.6^{a}	35.1 ± 3.5^{c}	75.9 ± 18.1^{ab}	47.3 ± 20.9^{bc}	66.7 ± 21.9^{bc}
	2º	20.0 ± 10.4^{b}	54.0 ± 2.5^{a}	77.5 ± 22.9^{a}	44.1 ± 2.4^{a}	50.9 ± 12.0^{a}	48.3 ± 25.8^{a}	64.7 ± 14.2^{a}
NO_3 (mg/L)	0	12.3 ± 2.0^{c}	101.7 ± 25.8^{b}	88.2 ± 25.3^{b}	56.4 ± 12.3^{b}	303.5 ± 92.2^{a}	74.3 ± 17.0^{b}	70.7 ± 20.2^{b}
	1º	10.7 ± 3.3b^{c}	21.0 ± 12.6^{abc}	25.4 ± 10.8^{ab}	16.0 ± 3.1^{abc}	32.4 ± 3.3^{a}	7.0 ± 1.5^{c}	33.0 ±18.1^{a}
	2º	45.3 ± 10.8^{a}	11.0 ± 10.8^{b}	8.7 ± 1.9^{b}	13.3 ± 6.1^{b}	6.3 ±1.6^{b}	10.5 ± 7.2^{b}	10.1 ± 1.7^{b}
PO_4 (mg/L)	0	4.3 ± 0.8^{a}	4.2 ± 0.1^{a}	4.1 ± 0.1^{a}	4.2 ± 0.1^{a}	4.7 ± 1.0^{a}	0.1 ± 0.04^{b}	0.03 ± 0.03^{b}
	1º	0.4 ± 0.3^{bc}	0.6 ± 0.5^{bc}	1.3 ± 0.8^{ab}	1.0 ± 0.3^{bc}	2.1 ± 0.9^{a}	0.1 ± 0.05^{c}	0.9 ± 0.5^{bc}
	2º	0.1 ± 0.1^{c}	0.8 ± 0.1^{a}	0.3 ± 0.3^{c}	1.3 ± 0.4^{a}	0.9 ± 0.3^{a}	0.6 ± 0.2^{ab}	0.4 ± 0.1^{bc}
SO_4 (mg/L)	0	171.4 ± 24.9^{cd}	654.9 ± 268.8^{ab}	582.9 ± 156.5^{b}	395.3 ±34.7^{cd}	875.0 ± 103.1^{a}	347.6 ± 40.9^{cd}	531.3 ± 100.2^{bc}
	1º	127.5 ± 21.0^{b}	271.8 ± 75.7^{a}	274.5 ± 73.1^{a}	102.7 ± 21.4^{b}	209.1 ± 99.8^{ab}	116.4 ± 28.6^{b}	285.5 ± 81.9^{a}
	2º	139.0 ± 17.7^{c}	247.0 ± 43.9^{a}	193.9 ± 21.3^{abc}	162.9 ± 8.6^{bc}	151.5± 38.3^{bc}	194.1 ± 73.5^{abc}	224.0 ± 48.7^{ab}

GW: *gossan* wastes (control); GA12 and GA30: Technosol containing GW and AgW+AW+RW at 12 and 30 g/kg; GC12 and GC30: Technosol containing GW and AgW+CW+RW at 12 and 30 g/kg; GAC12 and GAC30: Technosol containing GW and AgW+AW+CW+RW at 12 and 30 g/kg. AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops.
Different letters in data from the same row indicate significant differences ($p < 0.05$).

Amendment application (for some elements and except GA12) significantly reduced the concentrations of Al (> 36 %), Cu (GA12 > 16 % but for other treatments> 64 %), Pb (> 52 %), Fe (only GA30, GC12 and GAC30, ≈ 60 %) and nitrates (> 71 %) compared to control (1.8–3.0 mg Al/L, 0.4–0.5 mg Cu/L, 0.3–0.5 mg Pb/L, 0.2 mg Fe/L and 45.3 mg NO3/L). These reductions seem to be related to the increase of pH of leachates ($-0.65 < r < -0.85$). However, the application of organic matter can also promote the formation of complexes with cations and increase cationic exchange capacity (Adriano et al. 2004). According to Hodson et al. (2001), the immobilization of some metals (e.g. Pb) after addition of phosphate ions to contaminated soils can be related to the formation of metal phosphates with low solubility. The same was also reported by Santos et al. (2014c) in another type of mine wastes and using similar amendments. As the amendments used were a significant source of phosphates, this fact could also occur in GW. Moreover, small availability of As, Pb and Fe can be related to their presence in other solid phases presenting low solubility (Santos et al., 2012).

Cistus ladanifer germination and enzymatic activities

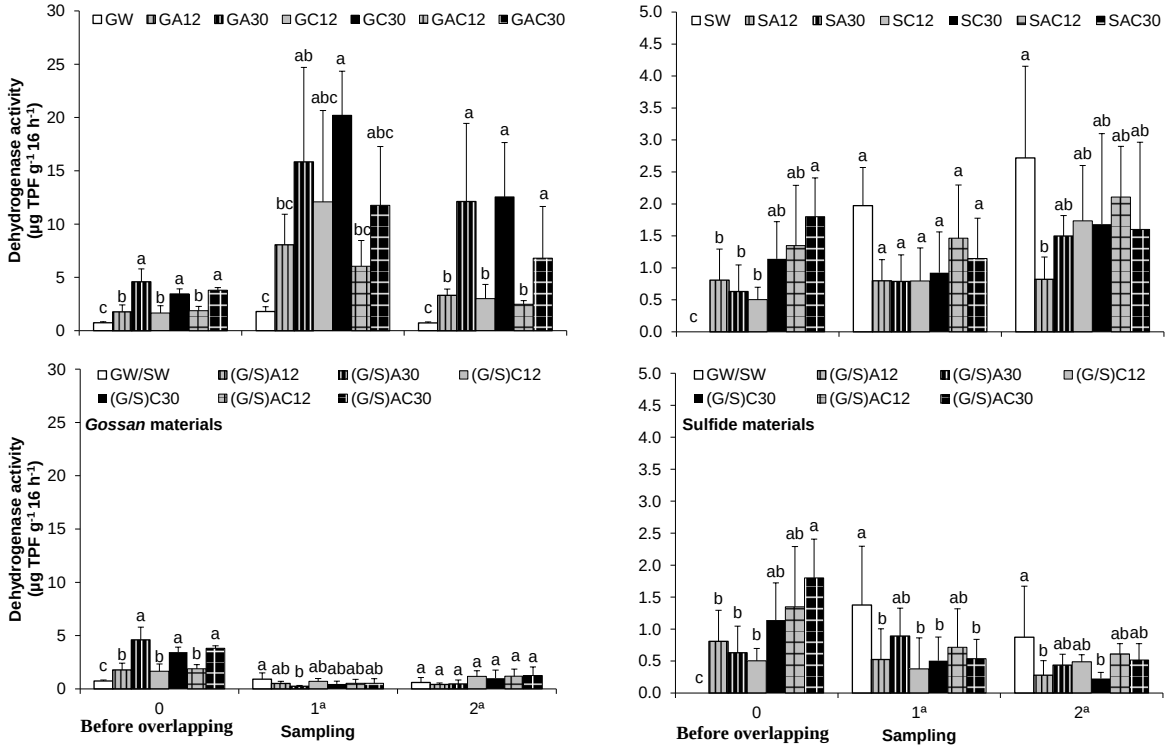
Germination and growth of *C. ladanifer* seedlings was stimulated in Technosols, compared to GW. Two months after sowing, a great variability in plant height was observed but, in general, the amendments provided nutrients which may explain the higher plant height. Thus, plants growing in Technosols had a height of 2–4 cm while in control they only had 1–1.5 cm. Nevertheless, no visible symptoms of deficiency or toxicity were observed in all seedlings. According to several authors (de Varennes et al., 2010a; Qu and de Varennes, 2010; Santos et al., 2014a), the improvement in the structure and water-holding capacity of soils developed on *gossan* wastes or even *gossan* wastes, by application of organic and inorganic wastes, are important factors that stimulate germination and growth of plants (*e.g. C. ladanifer*, *Spercularia purpurea* (Persoon) G. Don fil., *Briza maxima* L. and *Chaetopogon fasciculatus* (Link) Hayek). Moreover, the improvement in the fertility of these materials leads to higher plant biomass accumulation (de Varennes et al., 2010a,b; Qu and de Varennes, 2010; Santos et al., 2014a).

Enzymatic activities (Figs. 3 and 4) were used as biological indicators to assess the efficiency of the Technosols containing GW. General activity of microorganisms, evaluated by dehydrogenase activity, was stimulated in Technosols (1.66–20.20 μg TPF g^{-1} $16h^{-1}$) compared to control (0.72–1.79 μg TPF g^{-1} $16h^{-1}$) (Fig. 3). The same tendency was observed in the β-glucosidase activity (μmol ρ-nitrophenol g^{-1} h^{-1} – Technosols: 0.04–0.20, Control: 0.03–0.04; Fig. 5) but not in the acid phosphatase activity (μmol ρ-nitrophenol g^{-1} h^{-1} –Techonosols: 0.51–0.83, Control: 0.64–0.76; Fig. 4). The influence of organic matter on soil biological properties can vary with content, type, and dominant component of added organic materials (Tejada et al., 2006). However, in this assay, specially Technosols receiving a high dose of amendment mixture had higher ($p < 0.05$) dehydrogenase and β-glucosidase activities during the experiment (3.45–20.20 μg TPF g^{-1} $16h^{-1}$ and 0.13–0.20 μmol ρ-nitrophenol g^{-1} h^{-1}, depending on Technosol and sampling date) than the control (Figs. 3 and 4). According to Santos et al. (2014a) and de Varennes et al. (2010b), the significant increase of organic C by the application of high doses of amendment mixtures in GW or soils developed on *gossan*

wastes has a positive effect on dehydrogenase and β-glucosidase activities. This stimulation was also reported for acid phosphatase activity in soils developed on *gossan* (de Varennes et al., 2010b) in contrast to the results obtained in this study. Stimulation of dehydrogenase and β-glucosidase activities was also verified with addition of several organic and inorganic amendments to contaminated soils from other mine areas (Burgos et al., 2010; Pérez de Mora et al., 2005).

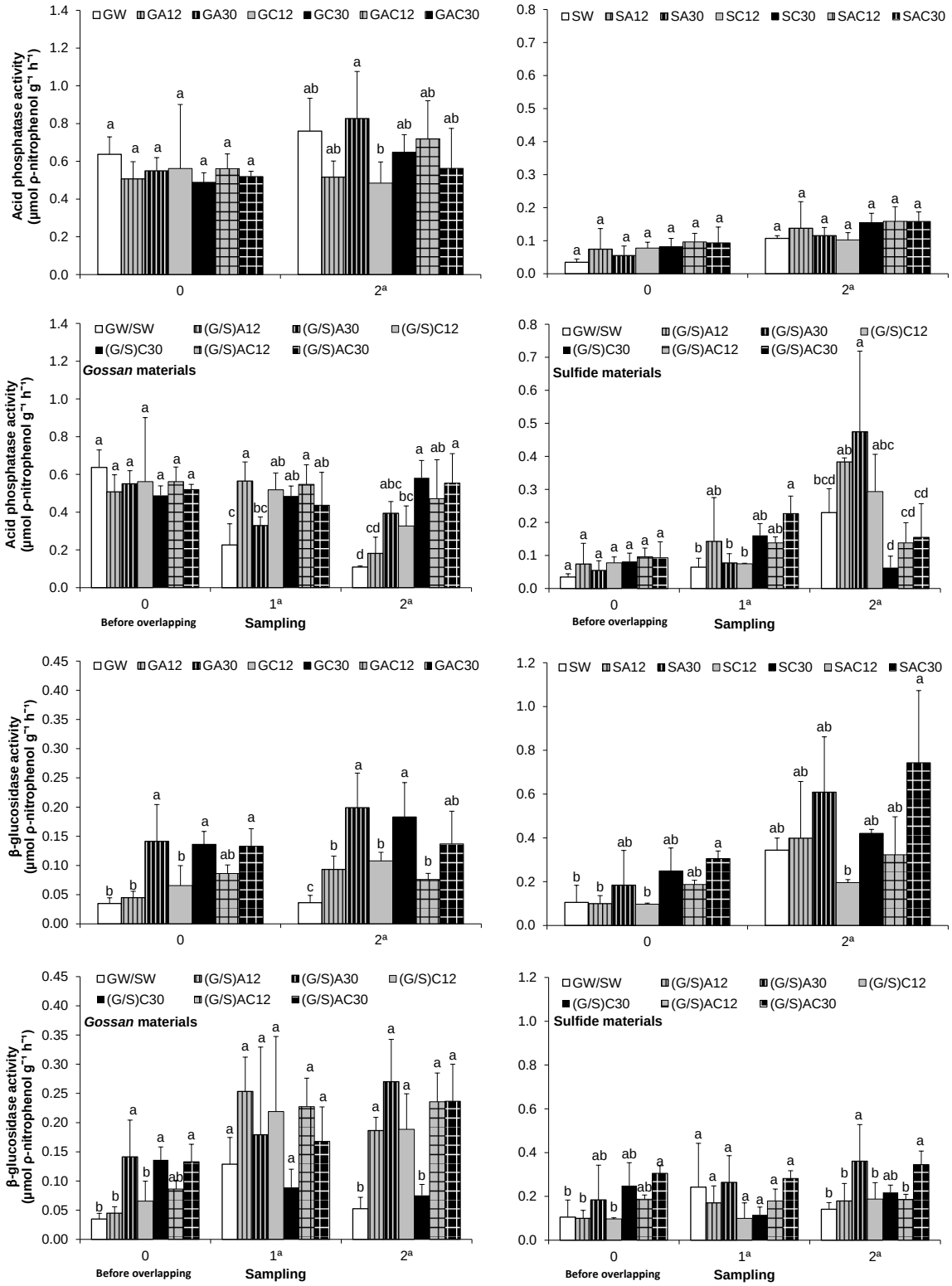
Although some authors (Burgos et al., 2010; Santos et al., 2014a) reported a correlation between pH of materials and enzymatic activities, no correlations were observed between pH or the concentration of elements in leachates and enzymatic activities in the present experiment.

The dehydrogenase activities of GW, GA12, GC30, GAC12 and GAC30 had an initial increase and then a decrease. Although in other treatments the same tendency was observed, the variations were not statistically significant due to the variability of the results. This suggests that amendments had a short lived impact on soil microorganisms.



GW: *gossan* wastes; SW: sulfide wastes; GW/SW: *gossan* waste layer over sulfide waste; GA12, GA30, SA12 and SA30: Technosol containing GW or SW and AgW+AW+RW at 12 and 30 g/kg; GC12, GC30, SC12 and SC30: Technosol containing GW or SW and AgW+CW+RW at 12 and 30 g/kg; GAC12, GAC30, SAC12 and SAC30: Technosol containing GW or SW and AgW+AW+CW+RW at 12 and 30 g/kg; (G/S)A12 and (G/S)A30: Overlapping of Technosols containing GW or SW and AgW+AW+RW at 12 and 30 g/kg; (G/S)C12 and (G/S)C30: Overlapping of Technosols containing GW or SW and AgW+CW+RW at 12 and 30 g/kg; (G/S)AC12 and (G/S)AC30: Overlapping of Technosols containing GW or SW and AgW+AW+CW+RW at 12 and 30 g/kg. AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops.

Fig. 3 Dehydrogenase activity in mine wastes and Technosols materials from the three assays (Mean ± SD; n = 3). Values from same assay and sampling period followed by a different letter are significantly different ($p < 0.05$).



GW: *gossan* wastes; SW: sulfide wastes; GW/SW: *gossan* waste layer over sulfide waste; GA12, GA30, SA12 and SA30: Technosol containing GW or SW and AgW+AW+RW at 12 and 30 g/kg; GC12, GC30, SC12 and SC30: Technosol containing GW or SW and AgW+CW+RW at 12 and 30 g/kg; GAC12, GAC30, SAC12 and SAC30: Technosol containing GW or SW and AgW+AW+CW+RW at 12 and 30 g/kg; (G/S)A12 and (G/S)A30: Overlapping of Technosols containing GW or SW and AgW+AW+RW at 12 and 30 g/kg; (G/S)C12 and (G/S)C30: Overlapping of Technosols containing GW or SW and AgW+CW+RW at 12 and 30 g/kg; (G/S)AC12 and (G/S)AC30: Overlapping of Technosols containing GW or SW and AgW+AW+CW+RW at 12 and 30 g/kg. AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops.

Fig. 4 Acid phosphatase and β-glucosidase activity in mine wastes and Technosols materials from the three assays (Mean ± SD; *n* = 3). Values from same assay and sampling period followed by a different letter are significantly different ($p < 0.05$).

Sulfide wastes

Chemical characterization of leachates

As expected, leachates from SW were more acidic (pH: 1.1–1.6; Fig. 1) and had higher ($p < 0.05$) electrical conductivity (more than 25-fold, 11.1–53.6 mS/cm; Fig. 1) and concentrations of elements (Table 4 and Fig. 5) than GW (Table 3 and Fig. 2). This fact is related to the high amount of sulfide minerals included in SW that contribute to the continuous formation of acid leachates and consequent release of chemical elements. Although different values of pH and electrical conductivity were obtained in the leachates from pyritic sludge (Pérez-López et al., 2007a,b; pH ≈ 2; EC: 2.5–4 mS/cm), in general, the concentration of chemical elements in SW leachates were in the same range as those reported for acid mine drainage in the IPB (Abreu et al., 2010; de la Torre et al., 2011; Ferreira da Silva et al., 2009; Sanchez-España et al., 2005, 2008).

The amendments increased significantly the pH of leachates (2.0–2.4), although these continued acidic, and reduced EC between 57 and 71 % compared to control (SW: 11.1–53.6 mS/cm) (Fig. 1). During the initial incubation (four months), the acidic nature of SW and the fine grain of the limestone rock wastes should promote a rapid dissolution of carbonates, but the other organic and inorganic wastes used had small buffering capacity so their application did not neutralize the leachates.

A slight increase in the pH of leachates (up to pH of 2.9) and reduction of electrical conductivity (more than 50 %) was also observed with the application of different doses of pig manure to acidic mine soils during 21 weeks (Garcés et al., 2008). However, mixture of fly ashes (waste with very high acid buffering capacity) with pyritic sludge mine was more effective in the improvement of pH and electrical conductivity of leachates between 4 and 7 months of incubation (pH from 2 to ≈7.5; EC from 2.5–4 to ≈0.5 mS/cm; Pérez-López et al., 2007a,b). Nonetheless, Herbert and Höckert (2009) also reported an increase of pH in leachates from waste rock from the Ljusnarsberg mine, which is composed of different sulfides, amended with paper mill sludge and activated sewage sludge between 120 and 400 days (from ≈3.5 to 6–7).

In general, concentrations of elements in leachates from all treatments were high at the beginning of the experiment and decreased over time (Table 4 and Fig. 5), with the same tendency as electrical conductivity. A similar behaviour was observed in leachates from pyritic sludge collected in São Domingos and waste rock from the Ljusnarsberg mine amended or not with different wastes (Herbert and Höckert, 2009; Pérez-López et al., 2007a,b). The intense formation of surface salts, namely metal-sulfates and Ca-phosphates, can explain the decrease in the concentrations of dissolved ions during the experiment (Santos et al., 2014c). Concentrations of K and Na from Technosols remained low during the experiment (Fig. 5) while leachates from control and Technosols with application rate at 12 and 30 g/kg and containing residue from the liquor distillation of A. unedo fruit (SA12 and SA30) collected in the first sampling date had a significant increase of phosphate (more than 10-fold; Table 4) compared to the beginning of the experiment.

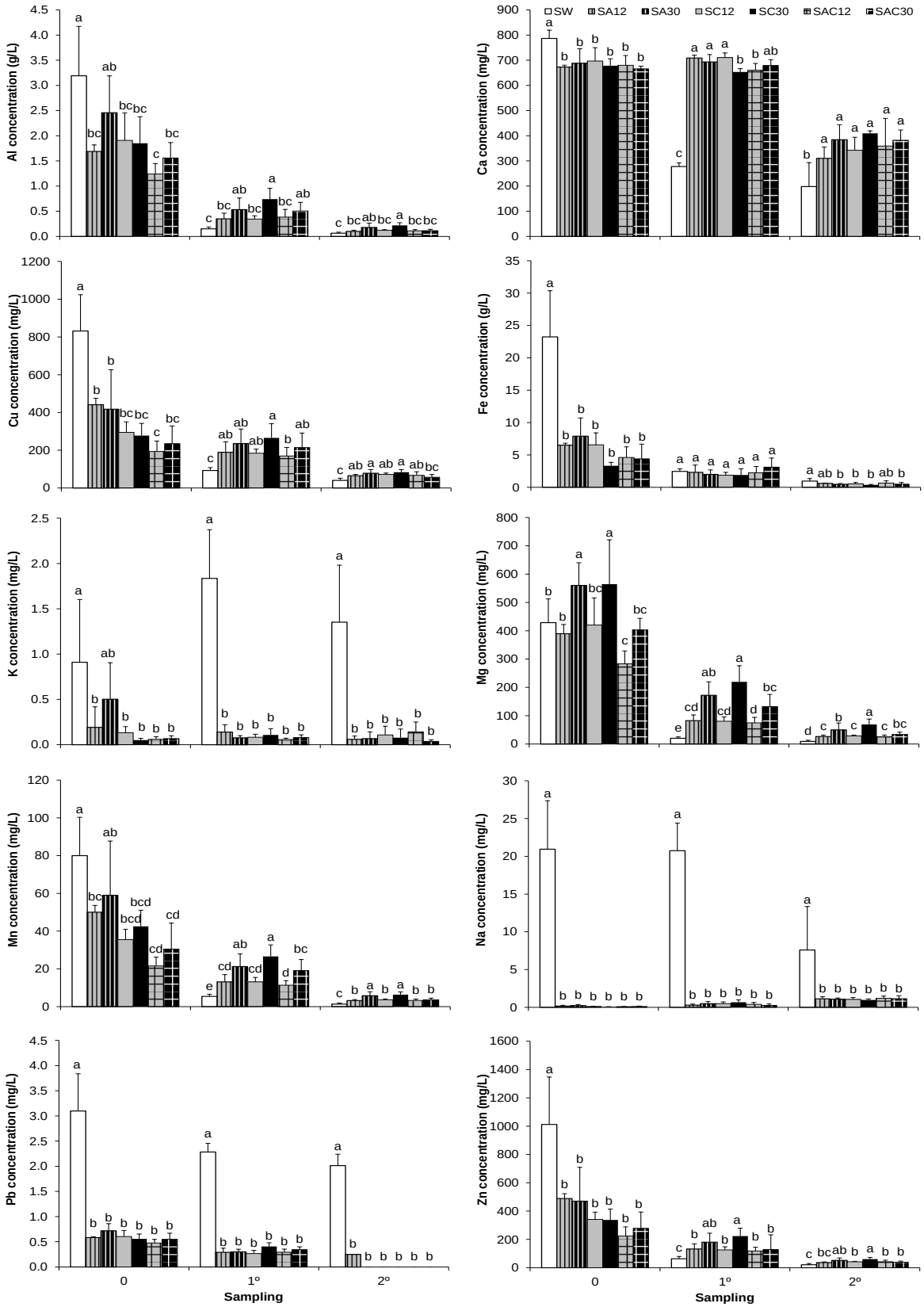
In the beginning of the experiment Technosols, independently of application dose and type of amendments, leached lower amounts of elements (except Mg and phosphate) than control (Table 4 and Fig. 5). However, this trend was not maintained for all chemical elements. Thus, during the

experiment, in leachates from all Technosols the concentrations of K, Na and Pb were reduced significantly by more than 85 % and that of sulfates at the end of experiment by 23–48 %, compared to control (1.4–1.8 mg K/L, 7.6–20.7 mg Na/L, 2.0–2.3 mg Pb/L, 5.9 g SO_4/L). Moreover, high application rates of amendment mixtures still led to a significant reduction of As (31–83 % depending on sampling period) and Fe (50–62 % at the end of the experiment) compared to control (9.3–14.5 mg As/L and 1.0 g Fe/L). The increase of cation exchange capacity, due to organic matter, can lead to the reduction of Pb, K and Na in solution. However, Garcés et al. (2008) reported a negative correlation between Pb concentration and dissolved organic carbon in leachates after application of pig manure to acidic mine soils. The decrease in the concentrations of elements can be also explained by the crystallisation of solid phases from alunite–jarosite-group and apatite–group as well as arsenbrackebuschite ($Pb_2Fe^{3+}(AsO_4)_2(OH)$) (Santos et al., 2014c).

Several authors (Herbert and Höckert, 2009; Pérez-López et al., 2007a,b; Sneddon et al., 2006) reported the reduction of the concentrations of several elements in leachates (*e.g.* As, Cu, Fe, Pb, Mn and Zn) following amendment application. However, Herbert and Höckert (2009) and Pérez-López et al. (2007b) referred that the application of some amendments (*e.g.* sewage sludge, fly ashes or paper mill waste + biosludge) to wastes containing sulfides did not lead to the immobilisation of some elements (*e.g.* Ca, K and Na) in leachates.

Technosols, independently of application dose and amendment type, leached higher ($p < 0.05$) amounts of Ca, Cu, Mg, Mn, Zn and phosphate (310–709 mg Ca/L, 56.9–265.4 mg Cu/L, 26.1–219.5 mg Mg/L, 3.2–26.6 mg Mn/L,36.0–223.3 mg Zn/L and 16.3–253.4 mg PO_4/L) than control (197.9–277.5 mg Ca/L, 40.3–92.6 mg Cu/L,9.4–20.3 mg Mg/L, 1.4–5.4 mg Mn/L, 21.1–62.9 mg Zn/L and 9.0–17.8 mg PO_4/L) in both sampling periods (Fig. 5). Concentrations of Al from the Technosols with high application rate of amendment mixture increased more than 2-fold being significantly different from the control (01–0.2 g Al/L). However, the coexistence of several elements in high concentrations can decrease the stabilization efficiency of the other elements due to, for example, competition for sorption sites (Kumpiene et al., 2008). The increase of Zn in leachates from Technosols can be related to an increase of dissolved organic carbon (Garcés et al., 2008) while the phosphates were linked to the organic matter and nutrient solution incorporated in the rockwool, which was used for strawberry cultivation. Nonetheless, the acidic conditions and the weathering of rockwool, which had significant concentrations of Al, may explain the increase of this element in leachates.

An increase of the amendments dose in sulfide wastes can modify significantly leachate characteristics but were not enough to prevent acid mine drainage. In conclusion, all leachates had large amounts of hazardous chemical elements representing a great environmental risk.



SW: sulfide wastes (control); SA12 and SA30: Technosol containing SW and AgW+AW+RW at 12 and 30 g/kg; SC12 and SC30: Technosol containing SW and AgW+CW+RW at 12 and 30 g/kg; SAC12 and SAC30: Technosol containing SW and AgW+AW+CW+RW at 12 and 30 g/kg. AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops.

Fig. 5 Concentrations of cations in leachates from Technosols and sulfide wastes – assay 2 (Mean ± SD; $n = 4$). Values from same sampling period followed by a different letter are significantly different ($p < 0.05$).

Table 4 Concentrations of anions in leachates of mine wastes and Technosols from assays 2 and 3 (Mean ± SD; *n* = 4) collected at time zero (0) and after three (1º) and nine (2º) months of incubation.

Anion	Sampling	Assay 2						
		SW	**SA12**	**SA30**	**SC12**	**SC30**	**SAC12**	**SAC30**
As (mg/L)	0	130.6 ± 93.3^{a}	1.6 ± 0.1^{b}	1.0 ± 0.5^{b}	1.2 ± 0.2^{b}	0.6 ± 0.5^{b}	1.7 ± 0.8^{b}	3.1 ± 2.4^{b}
	1º	14.5 ± 1.8^{a}	13.6 ± 2.2^{ab}	10.0 ± 2.7^{bc}	11.7 ± 1.9^{abc}	9.2 ± 3.1^{c}	10.7 ± 2.5^{ab}	8.3 ± 2.6^{c}
	2º	9.3 ± 2.7^{a}	10.4 ±2.3^{a}	1.6 ± 0.9^{b}	7.6 ± 4.1^{a}	1.8 ± 0.6^{b}	8.0 ± 2.8^{a}	1.8 ± 0.9^{b}
PO_4 (mg/L)	0	1.5 ± 0.3^{b}	0.9 ± 0.6^{b}	23.1 ± 0.2^{b}	86.7 ± 5.1^{a}	79.1 ± 6.3^{a}	80.5 ± 19.1^{a}	87.2 ± 12.4^{a}
	1º	17.8 ± 7.9^{c}	88.9 ± 33.4^{bc}	253.4 ± 119.9^{a}	102.4 ± 40.4^{bc}	126.6 ± 62.1^{b}	98.2 ±17.5^{bc}	84.4 ± 32.3^{bc}
	2º	9.0 ± 3.4^{d}	16.3 ± 1.9^{c}	34.3 ± 9.9^{a}	24.3 ± 12.7^{ab}	18.4 ± 4.9^{bc}	15.2 ± 4.0^{c}	31.0 ± 14.6^{ab}
SO_4 (g/L)	0	111.6 ± 24.2^{a}	36.9 ± 1.9^{b}	33.3 ± 12.4^{b}	28.1 ± 3.6^{b}	24.7 ± 4.1^{b}	21.8 ± 4.1^{b}	23.8 ± 7.5^{b}
	1º	18.6 ± 5.7^{a}	21.6 ± 4.4^{a}	21.4 ± 3.6^{a}	19.3 ± 2.9^{a}	23.1 ± 5.3^{a}	20.2 ± 2.5^{a}	22.8 ± 5.5^{a}
	2º	5.9 ± 1.7^{a}	3.7 ± 0.4^{b}	3.9 ± 1.9^{b}	4.3 ± 0.5^{ab}	4.5 ± 0.3^{ab}	3.3 ± 0.6^{b}	3.1 ± 1.4^{b}
		Assay 3						
		GW/SW	**(G/S)A12**	**(G/S)A30**	**(G/S)C12**	**(G/S)C30**	**(G/S)AC12**	**(G/S)AC30**
As	1º	2.2 ± 0.8^{a}	0.1 ± 0.1^{c}	0.1 ± 0.03^{c}	0.2 ± 0.03^{c}	0.1 ± 0.03^{c}	1.5 ± 0.6^{b}	1.1 ± 0.7^{b}
(mg/L)	2º	11.3 ± 3.7^{a}	2.9 ± 0.7^{c}	1.6 ± 0.6^{c}	2.4 ± 1.0^{c}	0.6 ± 0.3^{c}	7.3 ± 2.9^{b}	5.9 ± 2.0^{b}
PO_4	1º	94.4 ± 11.0^{c}	129.5 ± 57.4^{bc}	217.7 ± 124.2^{a}	185.1 ± 35.9^{ab}	68.8 ± 7.5^{c}	78.9 ± 21.0^{c}	62.4 ± 23.3^{c}
(mg/L)	2º	33.7 ± 7.3^{abc}	37.5 ± 14.4^{abc}	30.5 ± 2.3^{bc}	51.7 ± 26.7^{ab}	22.4 ± 9.8b^{c}	22.9 ± 10.3^{c}	54.6 ± 10.5^{a}
SO_4	1º	39.2 ± 5.4^{a}	25.8 ± 1.7^{b}	24.0 ± 2.2^{b}	26.6 ± 2.4^{b}	25.2 ± 9.5^{b}	26.8 ± 1.9^{b}	20.1 ± 5.9^{b}
(g/L)	2º	8.3 ± 1.0^{a}	6.4 ± 2.5^{a}	6.8 ± 0.6^{a}	5.2 ± 1.9^{a}	5.4 ± 1.0^{a}	6.9 ± 4.7^{a}	6.2 ± 2.0^{a}

SW: sulfide wastes; SA12 and SA30: Technosol containing SW and AgW+AW+RW at 12 and 30 g/kg; SC12 and SC30: Technosol containing SW and AgW+CW+RW at 12 and 30 g/kg; SAC12 and SAC30: Technosol containing SW and AgW+AW+CW+RW at 12 and 30 g/kg; (GW/SW): *gossan* wastes layer over sulfide wastes; (G/S)A12 and (G/S)A30: Overlapping of Technosols containing GW or SW and AgW+AW+RW at 12 and 30 g/kg; (G/S)C12 and (G/S)C30: Overlapping of Technosols containing GW or SW and AgW+CW+RW at 12 and 30 g/kg; (G/S)AC12 and (G/S)AC30: Overlapping of Technosols containing GW or SW and AgW+AW+CW+RW at 12 and 30 g/kg; AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo fruit*; CW: residue from liquor distillation of *C. siliqua fruit*; RW: rockwool used for strawberry crops.
Different letters in data from the same row indicate significant differences ($p < 0.05$).

Cistus ladanifer germination and enzymatic activities

No germination of *C. ladanifer* occured in any treatment probably due to the very acidic nature of SW and the continuous generation of acidity.

As expected, enzymatic activities were significantly smaller than those in GW (Figs. 3 and 4). In fact, less acidic conditions in contaminated soils can explain higher enzymatic activities, namely dehydrogenase (Burgos et al., 2010).

In the beginning of the experiment, amendments in some Technosols (SC30, SAC12 and SAC30) significantly stimulated dehydrogenase (1.14, 1.35 and 1.80 μg TPF g^{-1} $16h^{-1}$, respectively), compared to control where the activity was below the detection limit of the methodology (Fig. 3). Organic C derived from amendments may be responsible for the enhanced dehydrogenase activity in Technosols although dehydrogenase activity of control increased over time to similar values than that of Technosols (except SA12 in the 2nd sampling where dehydrogenase activity was lower than control). Variations of leachates characteristics did not correlate to dehydrogenase activity.

According to Hinojosa et al. (2008), toxicity of the pyrite sludge on dehydrogenase, acid phosphatase and β-glucosidase can be reduced by application of sugar-beet residues. However, no significant variations in acid phosphatase and β-glucosidase activities were detected with amendment application in this experiment (Fig. 4).

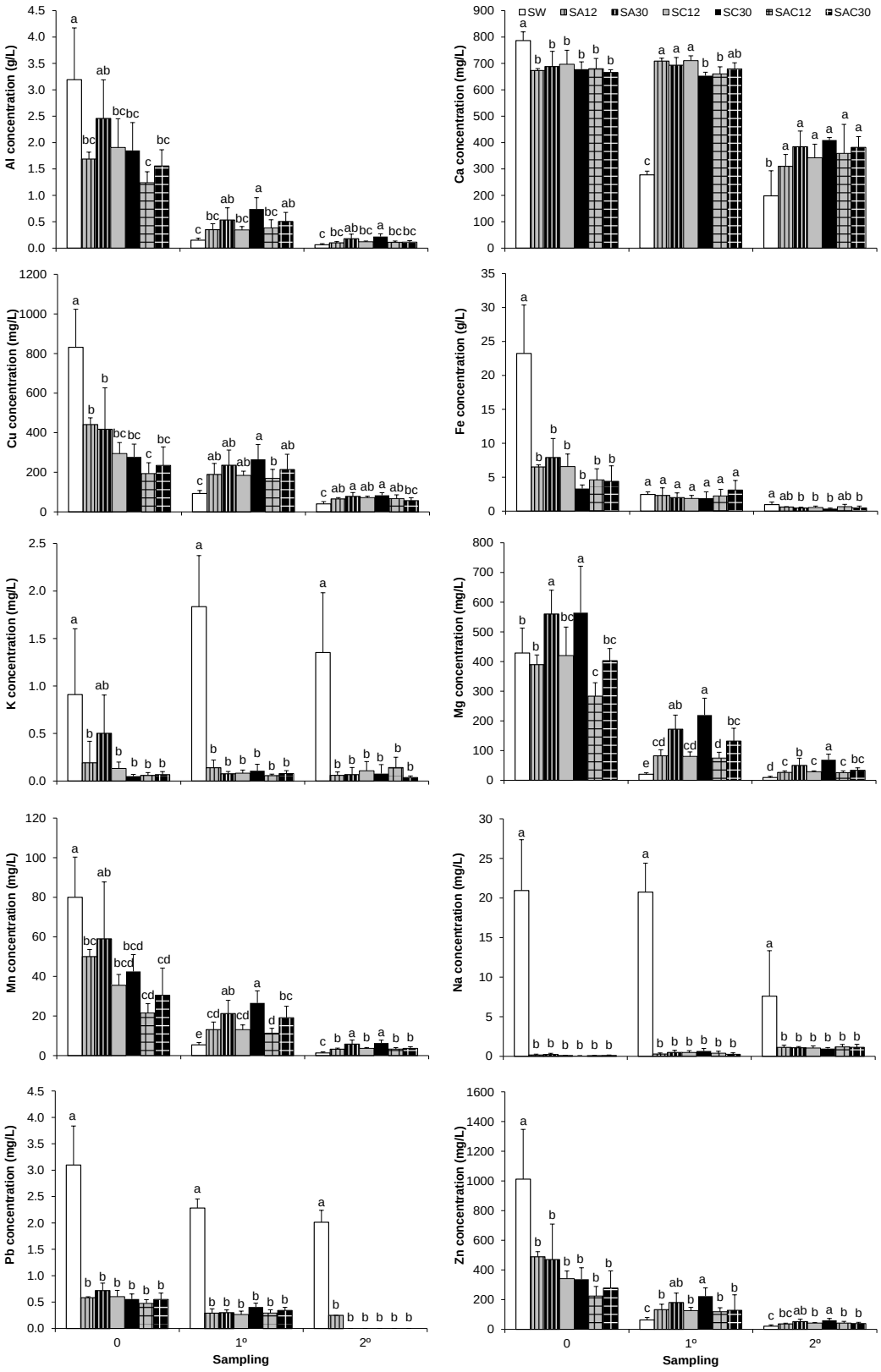
Sulfide wastes with a layer of gossan wastes

Chemical characterization of leachates

According to Pérez-López et al. (2007a,b), the main concern of sulfide wastes is related to acid mine drainage generation. In this assay, an attempt was made to isolate SW from air/water using a layer of GW which could support a pioneer vegetation which would reduce chemical elements leaching, promote microbial activity, ecological succession and soil formation.

Although leachates were still acidic and with high electrical conductivity, they had an electrical conductivity 36–60 % smaller and pH values increased more than 0.5 units, compared to control (GW/SW; 1.2< pH< 1.6 and 12.0–31.6 mS/cm; Fig. 1). Although application of *gossan* layer over SW seemed to reduce the EC of leachates, this fact also occurred in SW leachates, associated with a decrease of elements in solution over time. In general, application of a *gossan* layer over SW did not have the same effect than the superficial application of fly ashes where pH reached values ≈8.5 and EC was 0.5 mS/cm (Pérez-López et al., 2007a,b).

Leachates showed a reduction trend on the concentration of chemical elements (except for As and Ca) with time (Table 4 and Fig. 6). This reduction does not agree with the results obtained in leachates from SW covered by a superficial layer of fly ashes, where the elements concentrations were constant after four months of incubation (Pérez-López et al., 2007b).



SW: sulfide wastes (control); SA12 and SA30: Technosol containing SW and AgW+AW+RW at 12 and 30 g/kg; SC12 and SC30: Technosol containing SW and AgW+CW+RW at 12 and 30 g/kg; SAC12 and SAC30: Technosol containing SW and AgW+AW+CW+RW at 12 and 30 g/kg. AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops

Fig. 6 Concentrations of cations in leachates from Technosols and sulfide wastes – assay 2 (Mean ± SD; $n = 4$). Values from same sampling period followed by a different letter are significantly different ($p < 0.05$).

Technosols, independently of application dose and amendment type, leached higher concentrations of Mg in both sampling dates (54.2–354.5 mg/L) as well as Mn and Zn in the first sampling (29.9–45.6 and 252.0–372.3 mg/L, respectively) being significantly different from control (24.1–79.0 mg Mg/L, 16.4 mg Mn/L and 200.5 mf Zn/L) (Fig. 7). After that, significant differences were only obtained with high application doses ((G/S)A30, (G/S)C30, (G/S)AC30: ≈1 mg Mn/L). These differences were also observed for Al (2^{nd} sampling) where the leachates reached the highest concentrations (≈0.5 g Al/L). For Cu, Na and phosphate (in both or in one sampling) leachates from some Technosols reached higher concentrations than in control but with no clear tendency.

A reduction of more than 32 % was observed for As, Fe, K, Na and Pb in leachates, in both sampling periods, as well as sulfates at the end of the experiment (Table 4 and Fig. 6). Concentrations of As, Cu, Fe, Mn, Pb, Zn and sulfates in leachates also decreased with application of fly ashes over pyritic sludge (Pérez-López et al., 2007b).

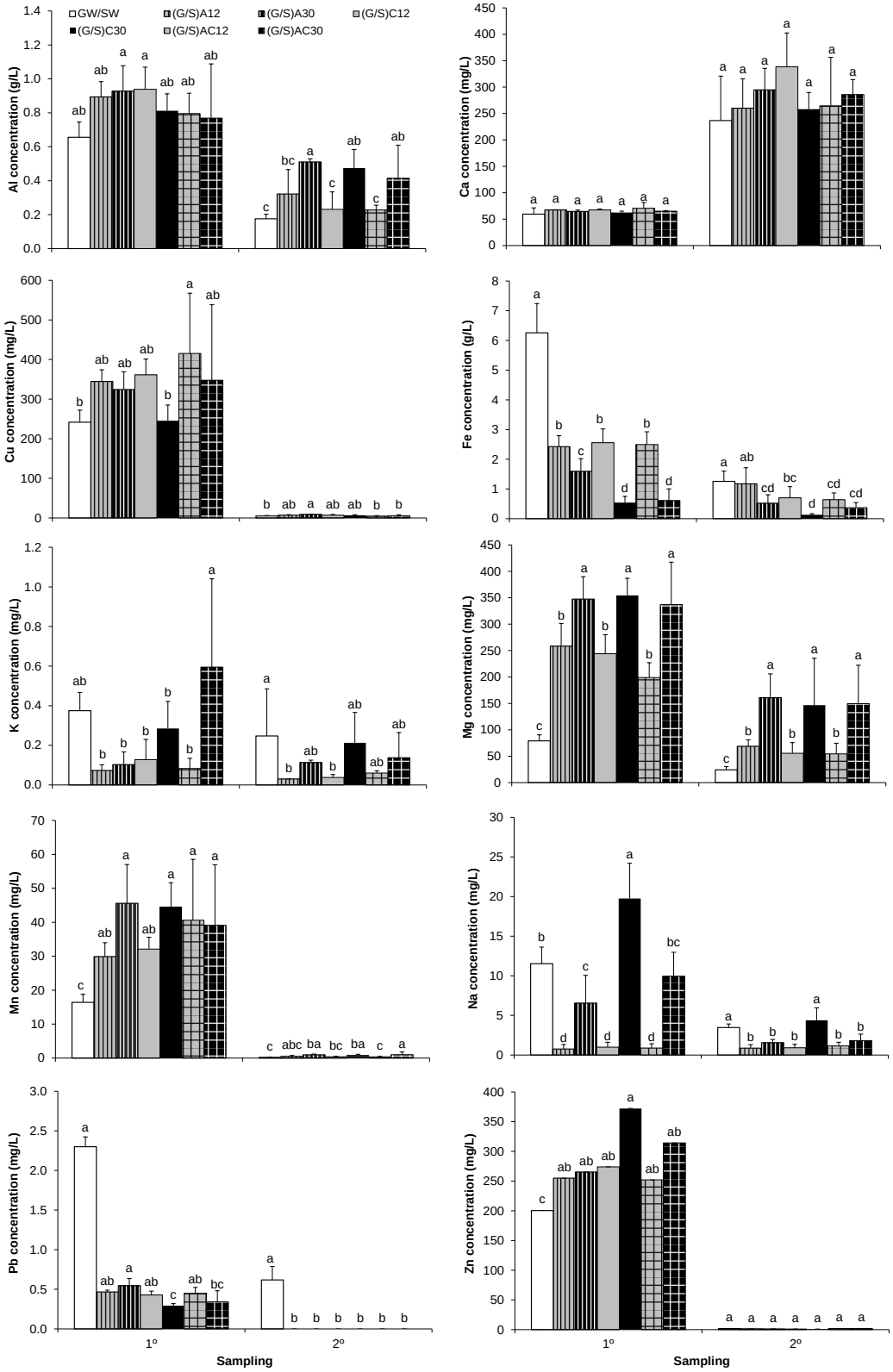
Although initially leachates went through the GW layer with a higher pH than the layer below, this did not neutralize the acidity (similar pH values than leachates from assay 2) or reduce the mobility of elements in SW. This fact may be related to the small thickness of the GW layer (≈4 cm) and eluviation of small particles of *gossan* materials and/or amendments to sulfide wastes. According to Santos et al. (2014c), the maintenance of humid SW (70 % of the maximum water-holding capacity) generates very acidic aqueous solutions (also observed in the present experiment – Fig. 1) containing several chemical elements, which then ascend to the surface by capillarity during the warm/hot months and crystallise due to water evaporation. The formation of small efflorescent soluble salts on the surface materials of this experiment confirms the occurrence of this process. The dissolution of these solid phases could also be observed in leachates as the concentration of elements and EC (Table 4 and Figs. 1 and 6) were significantly higher than those obtained in SW (Table 4 and Fig. 5).

Therefore, the overlapping of two Technosols or of two mine wastes (GW and SW) in the conditions tested was not sufficient to improve leachates from sulfide wastes.

Cistus ladanifer germination and enzymatic activities

Cistus ladanifer germination occurred only in Technosols. This fact does not agree with the results obtained with GW. The combined effect of a weak structure of *gossan* wastes and the acid solution flowing upwards by capillarity from SW may explain the total inhibition of seed germination in control. Approximately 50 days after sowing, plants had a height of 2–3 cm in Technosols with no visible symptoms of nutritional deficiency or phytotoxicity. However, after one week, all the seedlings died, coinciding with the emergence of small quantities of salt efflorescences on the surface. As Technosols seem to support the vegetation, a thicker layer of Technosols made with *gossan* materials and organic amendments may result in the rehabilitation of SW.

Enzymatic activities were determined in both materials (GW with/without amendments in the superficial layer, and SW with/without amendments in the deeper layer). In GW no differences were obtained among dehydrogenase activities of different treatments collected at both sampling periods (0.41–1.17 µg TPF g $16h^{-1}$ depending on treatment and sampling), as well as β-glucosidase activity in



(GW/SW): *gossan* wastes layer over sulfide wastes (control); (G/S)A12 and (G/S)A30: Overlapping of Technosols containing GW or SW and AgW+AW+RW at 12 and 30 g/kg; (G/S)C12 and (G/S)C30: Overlapping of Technosols containing GW or SW and AgW+CW+RW at 12 and 30 g/kg; (G/S)AC12 and (G/S)AC30: Overlapping of Technosols containing GW or SW and AgW+AW+CW+RW at 12 and 30 g/kg. AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops.

Fig. 7 Concentrations of cations in leachates from the overlapping of two Technosols or two mine wastes – assay 3 (Mean ± SD; $n = 4$). Values from same sampling period followed by a different letter are significantly different ($p < 0.05$).

the first sampling (0.09–0.53 µmol ρ-nitrophenol g^{-1} h^{-1} depending on treatment) (Figs. 3 and 4). Nevertheless, acid phosphatase activities and β-glucosidase activity of *gossan* materials from Technosols (collected in both sampling dates and at the end of the experiment, respectively) were significantly higher than control (µmol ρ-nitrophenol g^{-1} h^{-1} − GW/SW: 0.23 and 0.11 for acid phosphatase activities, 0.05 for β-glucosidase activity) (Fig. 4). As expected, chemical characteristics of leachates were not related to the enzymatic activities of the *gossan* materials. In fact, the pH and elements concentrations are the result of leaching through *gossan* and sulfide wastes or Technosols containing both mine wastes.

In all the treatments, no significant variations were obtained in dehydrogenase activity from *gossan* materials over time (Fig. 3). A similar tendency was observed for acid phosphatase, except in control (GW/SW) and Technosols containing residue from the liquor distillation of *A. unedo* fruit ((G/S)A12 and (G/S)A30), where acid phosphatase activity decreased ($p < 0.05$) during the experiment (Fig. 4). In contrast, β-glucosidase activities tended to increase in the first sampling (although only values from control, (G/S)A12 and (G/S)AC12 were significant) but remained constant afterwards (Fig. 4).

Dehydrogenase activities of *gossan* materials from all treatments were significantly smaller than those from the first assay; however, for other enzymatic activities the same behaviour only occurred in some treatments (acid phosphatase activity: GW/SW, (G/S)A12 and (G/S)A30; β-glucosidase activity: (G/S)C30 and (G/S)AC12). Acidic conditions and high concentrations of elements that ascend from SW can justify the reduction of the enzymatic activities. According to Hinojosa et al. (2008), pyrite sludge mixed with soil can affect soil enzymes through various pathways: activity of the produced enzyme, biosynthesis of microbial enzymes, and composition of soil microflora or microbial groups that produce extracellular enzymes.

Some amendment mixtures seemed to have an impact on enzymatic activities in sulfide materials; however, no clear tendency between dose and amendment type was observed. Thus in the 1st sampling, sulfide materials from some Technosols ((G/S)/A12, ((G/S)/C12, ((G/S)/C30 and ((G/S)/AC30) had lower ($p < 0.05$) dehydrogenase activities (<0.54 µg TPF g^{-1} $16h^{-1}$) than control (1.38 µg TPF g^{-1} $16h^{-1}$) while in the 2nd sampling the same happened in(G/S)/A12 and G/S)/C30 (µg TPF g^{-1} $16h^{-1}$ – ≈0.2; GW/SW: 0.87). In contrast, in the 2nd sampling, acid phosphatase and β-glucosidase activities of (G/S)/A30 (0.47 and 0.36 µmol ρ-nitrophenol g^{-1} h^{-1}, respectively) and acid phosphatase activity of (G/S)/AC30 (0.35 µmol ρ-nitrophenol g^{-1} h^{-1}) were significantly higher than in control (µmol ρ-nitrophenol g^{-1} h^{-1} – 0.23 and 0.14 for acid phosphatase and β-glucosidase activities, respectively).

In general, enzymatic activities were constant over time, independently of treatment. Although acid phosphatase activities of control, (G/S)/A30 and (G/S)/C12 increased between the first and second sampling periods, no correlation was established with chemical characteristics of leachates. Therefore, microbial functions of SW and Technosols containing SW did not change with the application of a superficial *gossan* layer with or without amendments.

CONCLUSIONS

Leachates from *gossan* wastes had low concentrations of elements and pH values around 3.5–4. In general, amendments of these wastes improved significantly the chemical characteristics of leachates and the biological activity of the materials and stimulated germination and plant growth. These facts are essential for the processes of natural attenuation or phytostabilization.

Chemical characteristics of leachates from sulfide wastes were typical of acid mine drainage, with very acidic pH (≈1) and high concentrations of anions and cations. Although electrical conductivity and elements (As, Fe, K, Na, Pb and SO_4) concentrations were reduced significantly and pH in leachates improved, amendments application was not enough to reduce sulfides oxidation or buffer leachates acidity. Dehydrogenase activity was also only stimulated in the beginning of the experiment while no alterations occurred in other enzymatic activities, suggesting that this amendment application is not an appropriate solution to remediate sulfide wastes.

Due to the great reactivity of sulfide wastes, their isolation from atmosphere may be the best solution for rehabilitation. Although the application of a thin superficial layer of *gossan* with or without amendments was not effective in the immobilization of chemical elements, some amendment mixtures seemed to have a positive effect on enzymatic activities and led to seed germination and seedling growth (although only during a short period), suggesting that a thicker layer of amended *gossan* materials may improve this system allowing plant establishment and, consequently, reducing chemical elements leaching from sulfide wastes.

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REFERENCES

Abreu, M.M., Batista, M.J., Magalhães, M.C.F., Matos, J.X., 2010a. Acid mine drainage in the Portuguese Iberian Pyrite Belt. In: Brock C.R. (Eds.), Mine drainage and related problems. Nova Science Publishers, Inc. New York, pp. 71-118.

Abreu, M.M., Santos, E.S., Ferreira, M., Magalhães, M.C.F., 2012a. *Cistus salviifolius* a promising species for mine wastes remediation. Journal of Geochemical Exploration 113, 86-93.

Adriano, D.C., 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability and risk of metals. New York, Springer-Verlag.

Alvarenga, P.M., Araújo, M.F., Silva, J.A.L., 2004. Elemental uptake and root leaves transfer in *Cistus ladanifer* L. growing in a contaminated pyrite mining area (Aljustrel-Portugal). Water, Air and Soil Pollution 152, 81-96.

Álvarez-Valero, A.M., Pérez-López, R., Matos, J., Capitán, M.A., Nieto, J.M., Sáez, R., Delgado, J., Caraballo, M., 2008. Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian Peninsula): evidence from a chemical and mineralogical characterization. Environmental Geology 55(8), 1797-1809.

Batista, M.J., Abreu, M.M., Serrano Pinto, M., 2007. Biogeochemistry in Neves Corvo mining region, Iberian Pyrite Belt, Portugal. Journal of Geochemical Exploration 92, 159-176.

Burgos, P., Madejón, P., Cabrera, F., Madejón, E., 2010. By-products as amendment to improve biochemical properties of trace element contaminated soils: effects in time. International Biodeterioration and Biodegradation 64, 481-488.

Corral, R., Pita, J.M., Pérez-García, F., 1990. Some aspects of seed germination in four species of *Cistus* L. Seed Science and Technology 18, 321-325.

de la Torre, M.L., Grande, J.A., Graiño, J., Gómez, T., Cerón, J.C., 2011. Characterization of AMD pollution in the River Tinto (SW Spain). Geochemical comparison between generating source and receiving. Water, Air and Soil Pollution 216, 3-19.

de Varennes, A., Cunha-Queda, C., Qu, G., 2010a. Amendment of an acid mine soil with compost and polyacrylate polymers enhances enzymatic activities but may change the distribution of plant species. Water, Air and Soil Pollution 208(1–4), 91-100.

de Varennes, A., Abreu, M.M., Qum G., Cunha-Queda, C., 2010b. Enzymatic activity of a mine soil varies according to vegetation cover and level of compost applied. International Journal of Phytoremediation 12(4), 371-383.

Eivazi, F., Tabatabai, M.A., 1977. Phosphatases in soils. Soil Biology & Biochemistry 9, 167-172.

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biology & Biochemistry 20, 601-606.

Ferreira da Silva, E., Bobos, I., Xavier, J.M., Patinha, C., Reis, A.P., Cardoso Fonseca, E., 2009. Mineralogy and geochemistry of trace metals and REE in volcanic massive sulfide host rocks, stream sediments, stream waters and acid mine drainage from the Lousal mine area (Iberian Pyrite Belt, Portugal). Applied Geochemistry 24, 383-401.

Freitas, H., Prasad, M.N.V., Pratas, J., 2004. Plant community tolerant to trace elements growing on degraded soils of São Domingos mine in the south east of Portugal: environmental implications. Environment International 30, 65-72.

Herbert, R.B., Höckert, L., 2009. Mine waste stabilization with biosludge and paper mill waste. Proceedings of 8th International Conference on Acid Rock Drainage and Securing the Future: Mining, Metals & the Environment in a Sustainable Society, 8 pp.

Hinojosa, M.B., Carreira, J.A., Rodríguez-Maroto, J.M., García-Ruíz, R., 2008. Effects of pyrite sludge pollution on soil enzyme activities: ecological dose–response model. Science of the Total Environment 396, 89-99.

Garcés, D.M.C., Cano, A.F., Arocena, J.M., 2008. Dissolved organic carbon and metals release in amended mine soil. Macla 10, 115-117.

Hodson, M.E., Valsami-Jones, E., Cotter-Howells, J.D., Dubbin, W.E., Kemp, A.J., Thornton, I., Warren, A., 2001. Effect of bone meal (calcium phosphate) amendments on metal release from contaminated soils - a leaching column study. Environmental Pollution 112, 233-243.

Kumar, S., Chaudhuri, S., Maiti, S.K., 2013. Soil dehydrogenase enzyme activity in natural and mine soil - a review. Middle-East Journal of Scientific Research 13(7), 898-906.

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments − a review. Waste Management 28, 215-225.

Macías, F., 2004. Recuperación de suelos degradados, reutilización de resíduos y secuestro de carbono. Una alternativa integral de mejora de la calidad ambiental. Recursos Rurais 1, 49-56.

Macías, F., Macías-García, F., Nieto, C., Verde, J.R., Pérez, C., Bao, M., Camps-Arbestain, M., 2011. Gestión de residuos y cambio climático. In: Mosquera, M.E.L., Osés, M.J.S., (Eds.), Gestión de residuos orgánicos de uso agrícola. Servizo de Publicacións e Intercambio Científico de la Universidade de Santiago de Compostela, Santiago de Compostela.

Matos, J.X., Martins, L.P., 2006. Reabilitação ambiental de áreas mineiras do sector português da Faixa Piritosa Ibérica: estado da arte e perspectivas futuras. Boletín Geológico y Minero 117, 289-304.

Pérez-de-Mora, A., Ortega-Calvo, J.J., Cabrera, F., Madejón, E., 2005. Changes in enzyme activities and microbial biomass after "in situ" remediation of a heavy metal-contaminated soil. Applied Soil Ecology 28, 125-137.

Pérez-López, R., Nieto, J.M., Almodóvar, G.R., 2007a. Immobilization of toxic elements in mine residues derived from mining activities in the Iberian Pyrite Belt (SW Spain): Laboratory experiments. Applied Geochemistry 22, 1919-1935.

Pérez-López, R., Nieto, J.M., Almodóvar, G.R., 2007b. Utilization of fly ash to improve the quality of the acid mine drainage generated by oxidation of a sulphide-rich mining waste: Column experiments. Chemosphere 67, 1637-1646.

Pérez-López, R., Álvarez-Valero, A.M., Nieto, J.M., Sáez, R., Matos, J.X., 2008. Use of sequential extraction procedure for assessing the environmental impact at regional scale of the São Domingos Mine (Iberian Pyrite Belt). Applied Geochemistry 23, 3452-3463.

Qu, G., de Varennes, A., 2010. Use of hydrophilic polymers from diapers to aid the establishment of *Spergularia purpurea* in a mine soil. Journal of Hazardous Materials 178, 956-962.

Quental, L., Bourguignon, A., Sousa, A.J., Batista, M.J., Brito, M.G., Tavares, T., Abreu, M.M., Vairinho, M., Cottard, F., 2002. MINEO Southern Europe environment test site. Contamination impact mapping and modelling - Final Report. Assessing and monitoring the environmental impact of mining activities in Europe using advanced Earth Observation Techniques (MINEO) 5yth FP-IST-1999-10337.

Rodríguez-Jordá, M.P., Garrido, F., García-González, M.T., 2012. Effect of the addition of industrial by-products on Cu, Zn, Pb and As leachability in a mine sediment. Journal of Hazardous Materials 213-214, 46-54.

Sánchez-España, J., Pamo, E.L., Santofimia, E., Aduvire, O., Reyes, J.A, Barettino, D., 2005. Acid mine drainage in the Iberian Pyrite Belt (Odiel river watershed, Huelva, SW Spain): Geochemistry, mineralogy and environmental implications. Applied Geochemistry 20(7), 1320-1356.

Sánchez-España, J., Toril, G.E., Pamo, E.L., Amils, R., Ercilla, M.D., Pastor, E.S., San Martín-Úriz, P., 2008. Biogeochemistry of hyperacid and ultraconcentrated pyrite leachate in San Telmo mine (Iberian Pyrte Belt, Spain). Water, Air and Soil Pollution 194, 243-257.

Santos, E.S., Abreu, M.M., Nabais, C., Saraiva, J., 2009. Trace elements and activity of antioxidative enzymes in *Cistus ladanifer* L. growing on an abandoned mine area. Ecotoxicology 18, 860-868.

Santos, E.S., Abreu, M.M., Nabais, C., Magalhães, M.C.F., 2012. Trace element distribution in soils developed on *gossan* mine wastes and *Cistus ladanifer* L. tolerance and bioaccumulation. Journal of Geochemical Exploration 123, 45-51.

Santos, E.S., Nabais, C., Abreu, M.M., 2013ª. Adaptabilidade ecofisiológica de diferentes populações de *Cistus ladanifer* L. do sul de Portugal. STUDIA – Scientiæ Rerum Diffusio: Suplemento Temático IV Seminário Luso-brasileiro em Ciências do Ambiente e Empresariais, 6 pp.

Santos, E.S., Abreu, M.M., de Varennes, A., Macías, F., Leitão, S., Cerejeira, M.J., 2013b. Evaluation of chemical parameters and ecotoxicity of a soil developed on *gossan* following application of polyacrylates and growth of *Spergularia purpurea*. Science of the Total Environment 461-462, 360-370.

Santos, E.S., Abreu, M.M., Macías, F., de Varennes, A., 2014a. Improvement of chemical and biological properties of *gossan* mine wastes following application of amendments and growth of *Cistus ladanifer* L.. Journal of Geochemical Exploration 147, 173-181.

Santos, E.S., Abreu, M.M., Batista, M.J., Magalhães, M.C.F., Fernandes, E., 2014b. Inter-population variation on the accumulation and translocation of potentially harmful chemical elements in *Cistus ladanifer* L. from Brancanes, Caveira, Chança, Lousal, NevesCorvo and São Domingos mines in the Portuguese Iberian Pyrite Belt. Journal of Soils and Sediments 14, 758-772.

Santos, E.S., Magalhães, M.C.F., Abreu, M.M., Macías, F., 2014c. Effects of organic/inorganic amendments on trace elements dispersion by leachates from sulfide-containing tailings of the São Domingos mine, Portugal. Time evaluation. Geoderma 226-227, 188-203.

Sneddon, I.R., Orueetxebarria, M., Hodson, M.E., Schofield, P.F., Valsami-Jones, E., 2006. Use of bone meal amendments to immobilise Pb, Zn and Cd in soil: A leaching column study. Environmental Pollution 144, 816-825.

Tabatabai, M.A., 1994. Soil enzymes. In: Mickelson, S.H., Bigham, J.M. (Eds.), Methods of soil analysis, Part 2, pp. 775-833.

Tejada, M., Hernández, M.T., García, C., 2006. Application of two organic amendments on soil restoration: effects on thesoil biological properties. Journal of Environmental Quality 35, 1010-1017.

Zhang, S., Li, W., Shan, X.Q., Lu, A., Zhou, P., 2005. Effects of low molecular weight organic anions on the release of arsenite and arsenate from a contaminated soil. Water, Air and Soil Pollution 167(1−4), 111-122.

9. IMPROVEMENT OF CHEMICAL AND BIOLOGICAL PROPERTIES OF GOSSAN MINE WASTES FOLLOWING APPLICATION OF AMENDMENTS AND GROWTH OF CISTUS LADANIFER L.

ABSTRACT

Gossan wastes represent one of the most hazardous mine wastes in several mining areas from the Iberian Pyrite Belt. Phytostabilization of mine wastes with *Cistus ladanifer* L. could be a good option, but its growth and germination are impaired by substrata conditions. To overcome these limitations, application of organic and inorganic amendments may speed up the growth of *C. ladanifer* while improving the mine wastes. This study evaluated the simultaneous influence of different application rates of amendments and growth of *C. ladanifer* on chemical and biological properties of *gossan* wastes.

Amendments used were mixtures (30, 75, 150 Mg/ha, 1:1:1) of rockwool, agriculture wastes and wastes from liquor distillation of *Arbutus unedo* L. fruits. A microcosm assay with four treatments was carried out (control and three amended treatments) under controlled conditions in a greenhouse during 505 days. C. ladanifer was sown in half of the pots from each treatment while the remainder was left bare.

Gossan wastes had large total concentrations of several elements (g/kg − Al: 24.8, As: 3.03, Cu: 0.23, Pb; 9.21) whereas the available concentrations of these elements were small (< 5.7 % of total). The amendments applied (in particular at 75 and 150 Mg/ha) improved the structure and increased the water-holding capacity, pH and nutrients concentrations in the available fraction of the *gossan* materials. They also led to increases in dehydrogenase and β-glucosidase activities and in plant growth (plant cover, plant height, length of young leaves, fresh biomass). In addition, plants from amended treatments presented lower concentrations of hazardous elements in shoots than plants from unamended control. The presence of the plant did not increase the available concentrations of hazardous elements in wastes, except for As when 150 Mg/ha of amendments were applied.

Phytostabilization with *C. ladanifer* using a Technosol, resulting from the application of the studied amendments at 75 and 150 Mg/ha to *gossan* materials seems a promising solution for rehabilitation of this type of mine wastes.

KEYWORDS São Domingos mine • Phytostabilization • Enzymatic activities

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INTRODUCTION

One of the major global environmental concerns in abandoned mining areas, namely in the Iberian Pyrite Belt (IPB), is related to the large volume of dispersed tailings, composed of different mine wastes (Abreu et al., 2010a; Matos and Martins, 2006; Tordoff et al., 2000). Mine wastes from Portuguese IPB have high total concentrations of several chemical elements and usually also acid-generating solid phases in some wastes (*e.g.* smelting ashes, milled pyrite) that can intensify their environmental impact, due to enhanced availability of chemical elements that can be transported by surface runoff to adjacent areas and/or leaching, and delay natural attenuation of mine wastes (Abreu et al., 2010a; Matos and Martins, 2006). Nevertheless, these mine tailings usually have sparse vegetation with slow natural colonization and scarce biodiversity.

The São Domingos mine (SE Portugal, Portuguese IPB) was exploited from the pre-roman period until 1960 decade, both in the *gossan* and volcanogenic massive sulfides ore deposits. Nowadays, this mine is abandoned and have large amounts of different mining wastes sparsely dispersed, which reach a total volume of 10.8×10^6 m^3, with high total concentrations of hazardous chemical elements (Álvarez-Valero et al., 2008; Matos and Martins, 2006). Considering the metal mobility, mass/volume and the bioavailable fraction of the potential contaminants, the *gossan* wastes represent the fourth most hazardous mine waste in São Domingos mine (modern slag > leaching tanks > country rocks > *gossan* wastes; Pérez-López et al., 2008), with approximately 1.7×10^6 m^3 of this material/residue (Álvarez-Valero et al., 2008).

Phytostabilization is considered an in situ green technology appropriate for the rehabilitation of mine wastes and multi-elemental contaminated soils in the Mediterranean basin (Abreu and Magalhães, 2009; EPA, 2000; Mendez and Maier, 2008; Tordoff et al., 2000). According to these authors, the importance of a plant cover is related to the improvement of the physical (*e.g.* structure), chemical (*e.g.* increase of organic matter and nutrients, immobilization of contaminants, decreased leaching) and biological (*e.g.* increase of microbial activity and diversity) characteristics of soil or wastes (Abreu and Magalhães, 2009 and references therein). Plants also restore ecosystem functions and lead to ecological succession, without requiring further disposal of the resulting biomass (EPA, 2000).

Cistus ladanifer L. is considered a good option for phytostabilization of mine wastes and soils developed on mine wastes as it is already present in several mining areas from the Portuguese IPB (Alvarenga et al., 2004; Batista et al., 2009; Freitas et al., 2004; Santos et al., 2009, 2012, 2014). However, this species grows slowly and has a small germination rate, due to the conditions of the mine substrata and Mediterranean climate (*e.g.* nutritional constraints, drought, intense irradiance, high total concentrations of trace elements), which are disadvantages for the success of phytostabilization.

Application of amendments can speed up the phytostabilization of the mine wastes (Abreu and Magalhães, 2009; Adriano et al., 2004; EPA, 2000; Macías, 2004; Macías et al., 2011). Several organic and inorganic wastes that can be used as amendments would otherwise end up in landfills (Macías, 2004; Macías et al., 2011). In general, the amendments improve substrata structure and

increase the nutrients and organic matter contents and water-holding capacity, essential characteristics in the rehabilitation of Mediterranean mining areas, and can also immobilize hazardous elements by several chemical processes (Adriano et al., 2004; Kumpiene et al., 2008; Tordoff et al., 2000), although some of these effects may not last long (Macías, 2004; Macías et al., 2011). In addition, mixtures of amendments should be adjusted to the conditions of each mine waste in order to produce a specific Technosol that promotes and maintains biogeochemical processes and reduces availability of contaminants to plants (Macías, 2004; Macías et al., 2011; Yao et al., 2009).

This study aimed to: evaluate the influence of combined use of different application rates of amendments and *C. ladanifer* growth on the improvement of chemical and biological properties of *gossan* wastes from the São Domingos mine; and assess the growth and accumulation of chemical elements of *C. ladanifer* during a phytostabilisation process.

MATERIALS AND METHODS

Experimental set-up

Composite samples (0−20 cm deep, nine subsamples, a total of approximately 90 kg) of mining wastes, which are composed of *gossan* materials mixed with host rocks (hereinafter named as *gossan* wastes − GW), and seeds from *C. ladanifer* (50 plants) were collected near of the open pit of São Domingos mine in 2009. This waste material was chosen due to the large amounts of trace elements, the corresponding potential environmental risk they pose (Álvarez-Valero et al., 2008; Pérez-López et al., 2008) and the fact that the area is already naturally colonized by autochthones plants.

The amendment applied was a mixture (1:1:1) of used rockwool from strawberry production in hydroponics, plant remains from several berries and substrate used in strawberry crops at 2:3 *m*/*m*, and wastes from liquor distillation of *Arbutus unedo* L. fruits (regional liquor). The proportion and type of organic and inorganic wastes used were chosen because of their physical and chemical properties and large quantities available near the mine at almost “zero cost”. These wastes are usually deposited in various locations without any treatment or legal permission.

The GW and amendment were air-dried before use. A microcosm assay was set up with four treatments (unamended control and application of the amendment at 30, 75 and 150 Mg /ha, each with six replicates) in pots containing about 2 kg of GW (fraction < 10 mm) mixed manually with the organic/inorganic wastes.

After 15 days of incubation at 70 % of the maximum water-holding capacity, *C. ladanifer* was sown (0.5 g seeds per pot) in half of the pots from each treatment (n = 3), while the other three pots remained bare. The seeds were pre-treated for optimal germination (heating at 100 °C for 30 min; Corral et al., 1990). All pots (with and without plants) were kept at 70 % of the maximum water-holding capacity in a greenhouse for 505 days.

Plants were thinned to 60 and 35 plants per pot 50 and 167 days after sowing, respectively, to minimize plant competition. Nitrogen was applied 280 days after sowing as an aqueous solution corresponding to 50 mg N/kg per pot in the form of calcium nitrate.

Experimental monitoring and analytical methods

The initial materials (fraction < 2 mm of GW and total fraction of organic and inorganic wastes) were characterized for pH and electrical conductivity (EC) in water suspension (1:2.5 *m*/*V*), organic C (Tinsley method), extractable P and K (Egner-Riehm method), total N (Kjeldahl method) and multi-elemental concentrations, which were analysed by atomic emission spectrometry with induced plasma and instrumental neutron activation analysis after acid digestion with $HClO_4$ + HNO_3 + HCl + HF (Actlabs, ISO/IEC 17025, Activation Laboratories, 2014). In GW (fraction < 2 mm), Fe from iron oxides (de Endredy, 1963) and Mn from manganese oxides (Chao, 1972) were also determined. Particle size distribution of GW (total fraction) was determined by sieving and sedimentation, with the clay fraction (< 2 μm) separated from coarser materials (< 2 mm) after water dispersion and sedimentation following Stokes law.

At the beginning (after 15 days of incubation and before sowing) and end of the experiment (505 days after sowing), composite samples of the materials from each pot were collected (0−15 cm of depth). These samples were homogenised and sieved (< 2 mm). A part of these samples were kept fresh (4 ΄C) for soil enzymatic analysis and determination of multi-elemental concentration in the available fraction while the remaining subsamples were air-dried for analysis of pH, EC, organic C, total N and extractable P and K, by the same methodologies referred above.

To determine the available fraction, elements were extracted from GW by the rhizosphere-based method, which uses a 10 mM mixture of organic acids (acetic acid + lactic acid + citric acid + malic acid + formic acid; Feng et al., 2005).

Several enzymatic activities were analysed as biological parameters to evaluate the rehabilitation process, namely: dehydrogenase (Tabatabai, 1994), β-glucosidase (EC 3.2.1.21; Eivazi and Tabatabai, 1988), acid phosphomonoesterase (acid phosphatase EC 3.1.3.2; Eivazi and Tabatabai, 1977) and urease (EC 3.5.1.5; Kandeler and Gerber, 1988). Dehydrogenase was used as an index of overall microbial activity while other enzymatic activities are related to C, P and N cycling.

The percentage of vegetation cover was determined 25 and 50 days after sowing, (Qu and de Varennes, 2010) and plant growth was analysed at each thinning (dominant plant height and length of young leaves and fresh biomass). At the end of the experiment, roots and shoots (composed of leaves and twigs) of *C. ladanifer* plants were collected. Plant samples were washed with tap water and then with distilled water. After washing, the roots were sonicated in distilled water for 30 minutes. Plant samples were dried (40 ºC), homogenised and finely ground. Elements (except N) were extracted from samples with ultrapure concentrated nitric acid (69 %) under pressure in a microwave digester during 2 min at 85 ºC + 5 min at 145 ºC + 5 min at 200 ºC + 18 min at 200 ºC. Total concentration of N was analysed by combustion with a Leco analyser. Certified reference samples of bush branches and leaves (NCSDC73348) and blanks were used in parallel to control the accuracy of the method.

The aqueous solutions from GW and plant extracts were stored at –18 ºC until analysed for Al, Ca, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn by flame atomic absorption spectrometry and As by graphite furnace atomic absorption spectrometry.

Data analysis

Data were analysed by a one way ANOVA and the Duncan test ($p < 0.05$) used to separate means, using the statistical programme SPSS v18.0 for Windows. For statistical purposes, the results below the detection limit were assumed as half of the detection limit. Bivariate Pearson correlations were used to correlate GW and plant characteristics ($r > 0.90$). Quality control of the analyses was made by analytical replicate samples (except for the total concentration of the chemical elements in GW and organic/inorganic wastes), use of certified standards solutions and reference plant samples, and laboratory standards at the international accredited laboratory (Activation Laboratories).

RESULTS AND DISCUSSION

Characterization of gossan mine wastes and amendments

Gossan wastes from the São Domingos mine had a clay loam texture, acid pH, small EC and a fertility characterised by low concentrations of organic C, total N and extractable P, but medium concentrations of extractable K (Table 1). These materials had great total concentrations of several potential hazardous elements (g/kg − Al: 24.8, As: 3.03, Cu: 0.23, Fe: 129; Pb: 9.21; Table 1) and could be considered as contaminated with As, Cu and Pb for commercial and industrial uses (CCME, 2007). Concentrations of some elements (e.g. As, Cu, Pb and Zn), as well as other chemical properties studied in this GW (Table 1), were in the same range as those reported for other *gossan* wastes from São Domingos (Álvarez-Valero et al., 2008; de Varennes et al., 2010; Pérez-López et al., 2008; Santos et al., 2012, 2013a).

The concentrations of elements in the available fraction were small varying between 0.01 and 5.7 % of the total concentrations, with the exception of Ca which was 23.2 % of the total concentration. Although the available fraction can change with time, space and the extraction solution used (Adriano, 2001; Kabata-Pendias, 2004), small concentrations of As, Cu, Fe, Pb and Zn in the available fractions were also obtained in same type of mine wastes from São Domingos by Pérez-López et al. (2008) and Santos et al. (2012). According to these authors, the presence of these elements mainly in the residual fraction, but also in iron and manganese oxides and/or organic matter can explain their low availability. In addition, the presence of solid phases in the residual fraction, with low solubility, like arsenates containing Pb and Pb+Fe can also contribute for their low availability (Santos et al., 2012).

All the wastes used as amendment had chemical properties considered useful and safe for land application and rehabilitation of GW. Values of pH and concentrations of total N, organic C and extractable P and K as well as total concentrations of several nutrients (Ca, Mg, Mn, P and Zn) in the amendments were larger than those in GW (Table 1). The C:N ratio depended on the type of residue (rockwool = 11; plant remains = 31; substrate used in strawberry crops = 60 and wastes from liquor distillation of *A. unedo* fruits = 9) suggesting different mineralization rates and release of N (de Varennes, 2003), which can be an advantage, in the medium-term, for the phytostabilization. Concentrations of hazardous elements in the amendments were much lower than those in GW, except

for Al in rockwool (Table 1). Although EC values of organic and inorganic wastes were higher than those in GW their application is without any environmental risk.

Table 1 Chemical characteristics of the *gossan* wastes from the São Domingos mine and of organic and inorganic wastes used as amendments**.**

	GW	**AgrW**	**ArbW**	**RW**
pH (H_2O)	4.32	6.68 – 7.18	4.91	7.08
EC (mS/cm)	<0.1	0.9 – 3.5	1.7	3.5
C organic (g/kg)	12.0	286.1	442.6	111.0
Total N (g/kg)	0.04	10.94 – 19.18	9.26	12.93
Extractable P (g/kg)	1.3×10^{-3}	0.34 – 3.15	0.18	7.92
Extractable K (g/kg)	0.08	0.17 – 6.56	3.57	0.73
Fe in iron oxides (g/kg)	74.32	--	--	--
Mn in Mn oxides (mg/kg)	3.00	--	--	--
	Total concentrations (g/kg)			
Al	24.8	4.06	1.37	53.4
As	3.03	2.3×10^{-3}	1.0×10^{-3}	$< 0.5 \times 10^{-3}$
Ca	0.5	22.56	10.30	139
Cu	0.23	44.6×10^{-3}	0.12	0.08
Fe	129	2.96	3.30	39.90
K	21.2	1.50	> 1.32	6.80
Mg	1.2	2.37	1.13	42.10
Mn	62.0×10^{-3}	0.25	51.2×10^{-3}	1.85
Na	1.7	0.31	0.40	13.10
P	1.08	NA	NA	6.63
Pb	9.21	5.05×10^{-3}	3.1×10^{-3}	$< 3 \times 10^{-3}$
S	13.7	NA	NA	2.60
Zn	36.0×10^{-3}	75.6×10^{-3}	27.3×10^{-3}	0.20

EC: Electrical conductivity; GW: *gossan* wastes; AgrW: Agriculture wastes (substrate used in strawberry crops - plant remains from several berries); ArbW: residue from the liquor distillation of *Arbutus unedo* L. fruit; RW: Rockwool

Characterization of the microcosm experiment

General chemical properties of the materials

At the beginning of the experiment (after 15 days of incubation and before sowing) and at the end of the experiment (505 days after sowing) amendment application improved the structure of GW (data not shown). They also increased the organic matter content and, consequently, the water-holding capacity. Values of pH from amended GW increased in both sampling periods (more than 0.4 units compared to the control) as well as the EC at the beginning of the experiment, compared to the control (Table 2). However, the EC values in amended GW were still small (< 748 µS/cm), compared to the application of other amendments like sewage sludge, sewage sludge+sugar beet sludge and

municipal solid waste compost in other contaminated soils (Alvarenga et al., 2008a,b). At small application rates, the reduction of EC with time can be related to leaching of the chemical elements in treatments without plants and uptake of elements when plants were present.

Table 2 Characteristics of *gossan* wastes from different treatments (control and materials with amendment applied at 30, 75 and 150 Mg/ha) at the beginning (after 15 days of incubation and before sowing) and end of the experiment (505 days after incubation with and without *Cistus ladanifer* growth) (mean ± SD; n = 3).

		Amended treatments		
	Control	**30 Mg/ha**	**75 Mg/ha**	**150 Mg/ha**
	After 15 days of incubation			
pH (H_2O)	3.74 ± 0.03^{cC}	4.10 ± 0.24^{bB}	4.46 ± 0.18^{bB}	5.22 ± 0.37^{aB}
EC (µS/cm)	194 ± 10^{cA}	365 ± 94^{bcA}	515 ± 284^{abA}	748 ± 57^{aA}
C organic (g/kg)	1.66 ± 0.22^{cA}	3.10 ± 0.61^{cC}	6.12 ± 0.60^{bC}	13.35 ± 4.42^{aA}
Total N (g/kg)	0.22 ± 0.06^{cA}	0.32 ± 0.06^{cA}	0.51 ± 0.04^{bB}	0.90 ± 0.27^{aA}
Extractable P (g/kg)	$< 0.02^{cA}$	0.16 ± 0.06^{bA}	0.09 ± 0.05^{bB}	1.15 ± 0.19^{aB}
Extractable K (g/kg)	1.51 ± 0.13^{bA}	1.77 ± 0.18^{bA}	3.09 ± 0.11^{aA}	2.63 ± 0.72^{aAB}
	After 505 days of incubation without plants			
pH (H_2O)	3.92 ± 0.03^{dB}	4.60 ± 0.31^{cA}	5.20 ± 0.21^{bA}	6.13 ± 0.04^{aA}
EC (µS/cm)	116 ± 10^{aB}	117 ± 16^{aB}	136 ± 17^{aB}	146 ± 47^{aB}
C organic (g/kg)	1.70 ± 0.87^{cA}	4.87 ± 0.85^{cB}	11.12 ± 1.98^{bB}	16.57 ± 3.00^{aA}
Total N (g/kg)	0.29 ± 0.07^{bA}	0.44 ± 0.17^{bA}	0.65 ± 0.25^{abA}	1.29 ± 0.04^{aA}
Extractable P (g/kg)	$< 0.02^{dA}$	0.48 ± 0.29^{bA}	0.13 ± 0.03^{cB}	1.08 ± 0.16^{aB}
Extractable K (g/kg)	1.45 ± 0.16^{bA}	1.88 ± 0.85^{bA}	3.07 ± 0.24^{aA}	3.55 ± 0.09^{aA}
	After 505 days of incubation with plants			
pH (H_2O)	4.07 ± 0.03^{cA}	4.58 ± 0.03^{bA}	5.52 ± 0.19^{aA}	5.77 ± 0.23^{aA}
EC (µS/cm)	155 ± 26^{bB}	176 ± 31^{bB}	231 ± 6^{bB}	577 ± 259^{aA}
C organic (g/kg)	2.29 ± 0.38^{cA}	7.48 ± 0.83^{cA}	15.44 ± 1.40^{bA}	22.09 ± 6.90^{aA}
Total N (g/kg)	0.23 ± 0.01^{cA}	0.43 ± 0.02^{cA}	0.81 ± 0.10^{bA}	1.22 ± 0.38^{aA}
Extractable P (g/kg)	$< 0.02^{cA}$	0.41 ± 0.32^{bA}	11.66 ± 2.93^{aA}	10.72 ± 2.76^{aA}
Extractable K (g/kg)	1.43 ± 0.16^{bA}	1.02 ± 0.19^{bA}	1.27 ± 0.11^{bB}	2.13 ± 0.41^{aB}

EC: Electrical conductivity.
Values for each parameter followed by a different letter are significantly different ($p < 0.05$). Small letters indicate comparisons between treatments from the same sampling date and capital letters indicate comparisons between treatments from different sampling dates.

The plants led to increased pH in control materials but were not enough to improve fertility, probably because so few survived in this treatment, and the ones that did remained small.

The increase of N, P, K and organic C was only significant with the highest application rates (75 and 150 Mg/ha), independently of the sampling period or presence of plants. At the end of the experiment, the fertility increased in the amended GW with the highest values observed in the treatments combining amendment and plants (especially for organic C at application rates of 30 and 75 Mg/ha and extractable P when amendment was applied at 75 and 150 Mg/ha).

In general, the amendment, especially at the highest application rates, and *C. ladanifer* growth enhanced much more the pH and fertility of this type of GW than polyacrylate polymers and *S. purpurea* (Santos et al., 2013a).

Available fraction of the elements in the materials

In both sampling periods, the concentration of Pb was close or below 5 mg/kg, the detection limit of the apparatus. Similar results were obtained by Alvarenga et al. (2008b) in contaminated soils from the Aljustrel mine amended with different wastes.

Amendment application, especially at high application rates (75 and 150 Mg/ha) led to increases in the concentrations of other hazardous elements (Al and As) and nutrients (except Cu and Zn in the beginning and end of the experiment in the presence of plants) in the available fraction in both sampling periods and independently of the plant (Fig. 1). These high available concentrations are related to the incorporation of rockwool, which had Al in its composition, and nutrients coming from the remaining nutrient solution used to grow strawberry (Table 1). However, no relationship was obtained between pH and organic C and the available concentrations of the elements. Similar results were obtained by several authors using the same or other extraction methods (Alvarenga et al., 2008a,b; Burgos et al., 2010; Santos et al., 2013a).

In general, the available concentrations of Al, Ca, Fe, K and Mg decreased with time in the absence of plants (Fig. 1), although with no clear trend. Similar results were reported for Cu, Mn and Zn with application of different amendments and acid soil by Burgos et al. (2010). Low concentrations of these elements at the end of the experiment, compared with the beginning, can derive from chemical reactions and leaching during the experiment. Additionally, application of organic matter can promote the formation of complexes with cations (although no relationship was obtained in the present study) and increase cationic exchange capacity (Adriano et al., 2004).

In contrast, the concentration of As (at an application rate of 30 Mg/ha) and Cu and Zn (at application rates of 75 and 150 Mg/ha) at the end of the experiment were higher than in the beginning. In fact, a considerable fraction of Cu and Zn can be associated with organic matter in the *gossan* materials (Abreu et al., 2010b; Santos et al., 2012), and the mineralization of the organic matter could promote the release of these metals. However, no relationship was obtained between available concentrations of these metals and increases in organic C with amendment application rate. Inhibition of the formation of low solubility solid phases (*e.g.* metal arsenates) could have occurred (Abreu et al., 2009; Santos et al., 2012).

At the end of the experiment, the presence of plants did not influence the concentrations of Al, Ca, Fe, Mg and Mn in the available fraction of GW, independently of the treatment (Fig. 1). However, the concentration of some nutrients decreased with plant growth, namely K in all amended treatments, Cu in the amended treatment at 150 Mg/ha and Zn in the amended treatments at 75 and 150 Mg/ha. This could be related to uptake by plants or more exudation of organic acids by roots, capable of forming complexes and reducing the availability of the cations (Feng et al., 2005; Gonzaga et al., 2012; Tu et al., 2004).

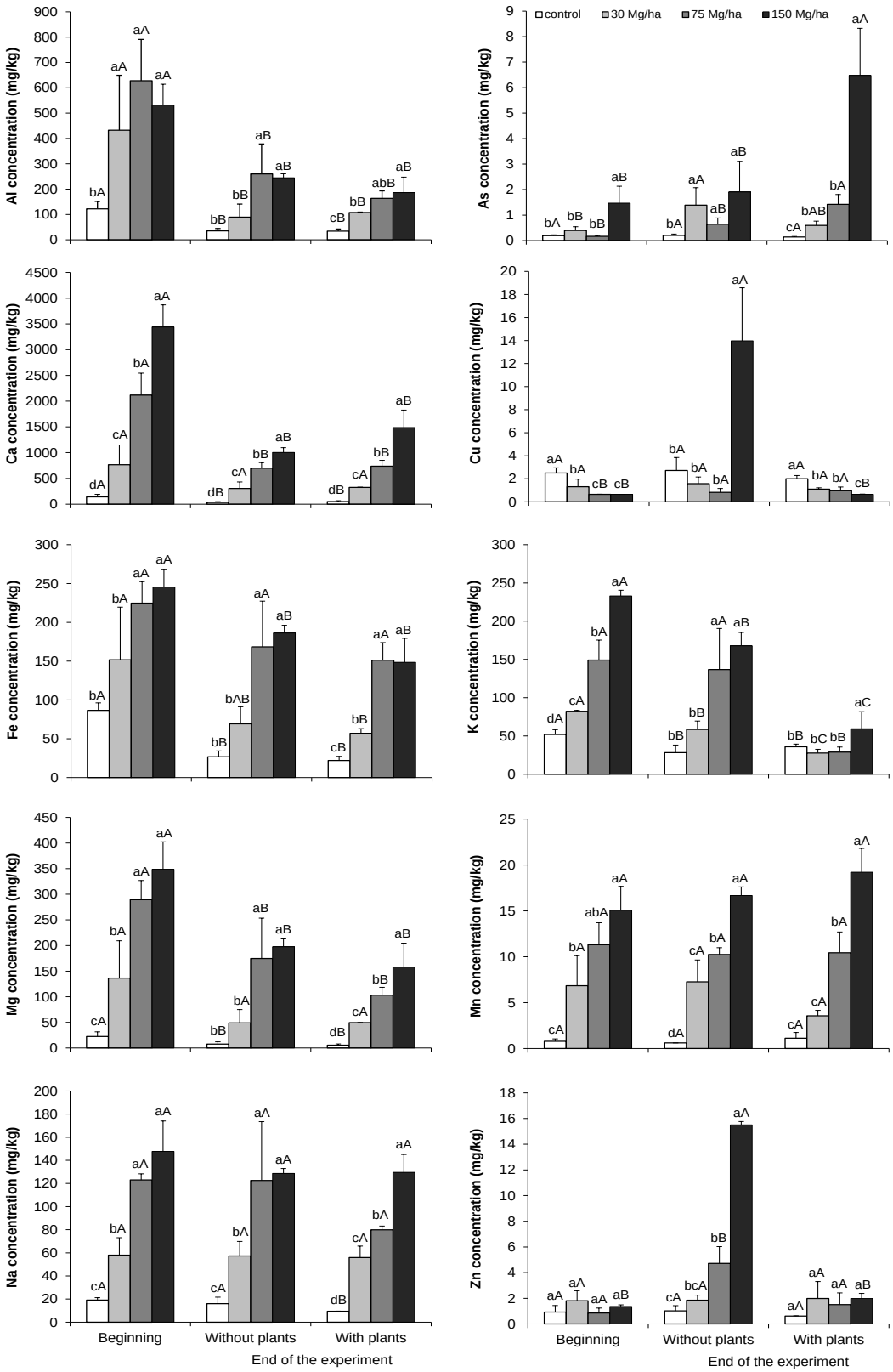

Fig. 1 Effects of treatments (control and *gossan* wastes with amendment application at 30, 75 and 150 Mg/ha) and presence of *Cistus ladanifer* on the concentration of chemical elements in the available fraction of *gossan* wastes at the beginning (after 15 days of incubation and before sowing) and end of the experiment (505 days after sowing) (Mean ± SD; $n = 3$). Values followed by a different letter are significantly different ($p < 0.05$), small letters indicate comparisons between treatments from the same sampling date and capital letters indicate comparisons between treatments in different dates and with or without plants.

The presence of plants in treatments with higher amendment application rates (75 and 150 Mg/ha) increased by 2 and 3-fold the available As in GW, respectively. The same behaviour was observed in a mesoscosm experiment using soils developed on *gossan* with different types of polyacrylate polymers and *S. purpurea* and natural plant colonization (Santos et al., 2013a). The increase of dissolved organic carbon in the rhizosphere due to exudation of low-molecular-weight organic acids by roots as well as changes in the composition of root exudates may explain the high As availability (Feng et al., 2005; Gonzaga et al., 2012; Tu et al., 2004). Thus, high application rates of amendments can contribute to an increase of low-molecular-weight organic acids and affect the availability of As. Organic acids can also form complexes with cations or block As adsorption sites on soil particles as iron oxides (Feng et al., 2005; Gonzaga et al., 2012; Tu et al., 2004). Root exudates of *Pteris vittata* L. (an As hyperaccumulating Chinese Brake fern) and Nephrolepis exaltata L. (an As-sensitive Boston fern) dissolved significant amounts of As from two arsenates (Tu et al., 2004). Low-molecular-weight organic acids can also release As from Fe−, Mn− and Al−oxides or hydroxides in contaminated soils (Zang et al., 2005).

Although GW had large total concentrations of some elements (*e.g.* Al, As, Cu and Pb), the concentrations potentially available for organisms (*C. ladanifer* and microorganisms involved in biogeochemical cycles) after amendment application represented a low potential environmental risk, with the increase of nutrient availability being an advantage for the rehabilitation process.

Enzymatic activities in the materials

Enzymatic activities (Fig. 2) were used as biological indicators to monitor the phytostabilization of GW. The smallest values of dehydrogenase and β-glucosidase activities were obtained in the control (0.71-1.78 µg TPF g^{-1} $16h^{-1}$ and 0.03-0.05 µmol ρ-nitrophenol g^{-1} h_{-1}, respectively). However, urease and phosphatase activities in the same treatment (0.81-1.14 µmol N-NH_4^+ g^{-1} $2h^{-1}$ and 0.64-1.06 µmol ρ-nitrophenol g^{-1} h^{-1}, respectively) were similar or even higher than in the amended treatments (< detection limit-0.91 µmol N-NH_4^+ g^{-1} $2h^{-1}$ and 0.51-1.17 µmol ρ-nitrophenol g^{-1} h^{-1}, respectively), depending on sampling date. Only phosphatase and β-glucosidase activities were influenced positively (≈1.4-fold greater) by the presence of plants. A similar behaviour for all enzymatic activities, except β-glucosidase, was reported in a microcosm assays where *gossan* materials from the São Domingos mine were also used and where *Briza maxima* L., *S. purpurea* and *Chaetopogon fasciculatus* (Link) Hayek were present (de Varennes et al., 2010).

In general, soil microorganisms, evaluated by the dehydrogenase activity, benefited from the presence of plants and amendment application, especially at higher application rates (75 and 150 Mg/ha). Increase of this activity was also verified with addition of several organic and inorganic amendments to contaminated soils with and without plants (Alvarenga et al., 2008b; Burgos et al., 2010). The β-glucosidase activity was also greater in amended GW compared to control, but the presence of plants did not affect its activity over that of bare GW. An increase in organic C and pH in the amended GW can explain the highest activities of dehydrogenase ($0.95 < r < 0.97$ and $0.85 < r < 0.93$, depending on sampling period and presence of plants) and β-glucosidase ($0.90 < r < 0.98$ and

0.90 < *r* < 0.94, depending on sampling date). The effect of these two soil parameters in β-glucosidase activity was also reported by Alvarenga et al. (2008a).

For phosphatase and urease activities, no clear tendency was observed between treatments in agreement with the results obtained by the application of some organic and inorganic wastes combined with plant growth (*L. perenne* and *L. multiflorum*) in contaminated soils from the Aljustrel mine (Alvarenga et al., 2008a,b),

In general, a positive effect of root exudates, availability of nutrients and organic C and/or pH increase derived from amendment application to *gossan* materials (especially with high application rates) on dehydrogenase and other soil enzymes related to nutrient cycling was observed.

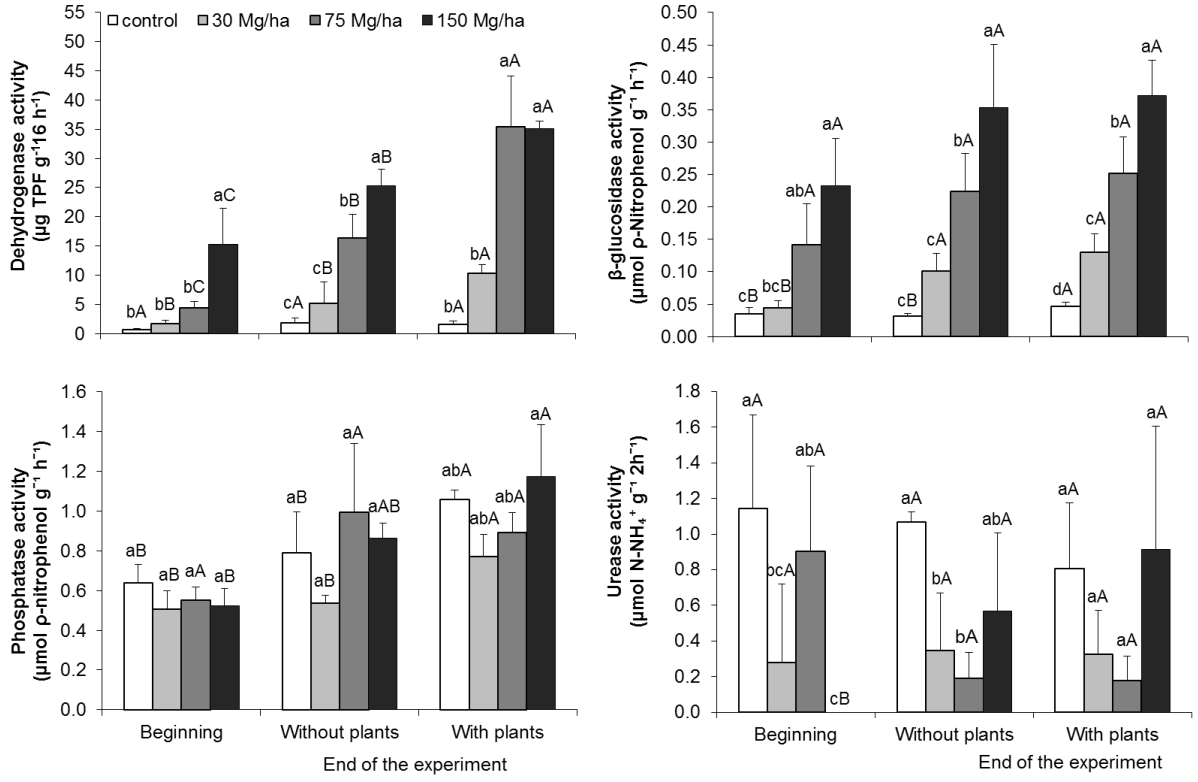

Fig. 2 Effects of treatments (control and *gossan* wastes with amendment application at 30, 75 and 150 Mg/ha) and presence of *Cistus ladanifer* on enzymatic activities at the beginning (after 15 days of incubation and before sowing) and end of the experiment (505 days after sowing) (Mean ± SD; *n* = 3). Values followed by a different letter are significantly different ($p < 0.05$), small letters indicate comparisons between treatments from the same sampling date and capital letters indicate comparisons between treatments in different dates and with or without plants.

Growth of Cistus ladanifer

There were no significant differences between values of plant cover 25 days after sowing (Table 3), but by 50 days after sowing the plant cover reached the highest values in the treatment 150 Mg/ha. The opposite was observed for the same species sown in a soil developed on *gossan* materials and amended with hydrophilic polyacrylate polymers (Santos et al., 2013a).

Table 3 Effects of treatments (control and *gossan* wastes with amendment applied at 30, 75 and 150 Mg/ha) on plant cover 25 and 50 days after sowing (minimum – maximum; mean; $n = 3$).

		Amended treatments		
Ground cover (%)	**Control**	**30 Mg/ha**	**75 Mg/ha**	**150 Mg/ha**
25 days	0 − 31	9 − 19	0 – 23	2 − 47
	14[a]	12[a]	10[a]	32[a]
50 days	55 – 86	83 – 96	92 − 93	96 − 98
	77[b]	91[ab]	93[ab]	97[a]

Values for each date followed by a different letter are significantly different ($p < 0.05$).

No relationship was observed between the properties of GW and plant cover. However, the improvement of structure and water-holding capacity, due to the incorporation of rockwool and organic wastes, may explain the larger area covered by *C. ladanifer* in amended treatments. Wang et al. (2008) reported that an unfavourable physical structure in tailings from the Xiangtan mine was the most significant constraint for the establishment of natural vegetation. In addition, the improvement of water-holding capacity by hydrophilic polyacrylate polymers application to soils developed on *gossan* also enhanced soil cover and growth of *S. purpurea*, *B. maxima* and *C. fasciculatus* (de Varennes et al., 2010; Qu and de Varennes, 2010).

Plants were taller, accumulated morefresh biomass in roots and shoots, and had larger young leaves in the amended GW than in control; their survival rate was also greater (Amended treatments: 66−89 %; Control: 66−89 %) (Tables 4 and 5). However, in general, no significant differences among amended treatments were observed. During all the experiment, the higher available concentrations of nutrients and organic C in GW by amendment application (Fig. 1 and Table 2) led to better growth of *C. ladanifer* (Tables 4 and 5) in agreement with the reported by several authors using different amendments, contaminated soils/mine wastes and plant species (Alvarenga et al., 2008b; Conesa et al., 2007; de Varennes et al., 2010; Qu and de Varennes, 2010; Santos et al., 2013a). Moreover, no negative influence of the hazardous elements in the GW available fraction on growth of *C. ladanifer* was observed.

The reduction of the root-shoot ratio (dry weight of root biomass/dry weight of shoot biomass) from 0.6 in control to 0.3−0.4 in amended treatments, also suggests a plant response to more favourable growing conditions (Harris, 1992). In this way, improvement of the fertility of GW was of major importance for the success of phytostabilization, especially in early stages of plant growth (Conesa et al., 2007; Tordoff et al., 2000).

Table 4 Effects of treatments (control and *gossan* wastes with amendment applied at 30, 75 and 150 Mg/ha) on *C. ladanifer* growth 50, 167 and 505 days after sowing (minimum – maximum; mode; *n* = 3).

		Amended treatments		
Plant parameters (cm)	**Control**	**30 Mg/ha**	**75 Mg/ha**	**150 Mg/ha**
		50 days		
Plant height	1.0 – 1.5	1.5 – 4.0	1.0 – 3.0	1.0 – 4.0
	1.0	3.0	2.0	2.0
Young leaves length	0.5 – 0.7	0.5 – 1.5	1.0 – 2.0	1.0 – 1.5
	0.5	1.0	1.0	1.0
		167 days		
Plant height	1.5 – 3.0	3.5 – 6.0	4.0 – 6.5	4.5 – 7.0
	2.5	3.5	4.5	5.0
Young leaves length	0.6 – 1.0	0.8 – 1.0	1.0 – 2.0	1.5 – 2.5
	0.6	1.0	1.0	1.5
		505 days (end of the experiment)		
Plant height	3.0 – 7.0	7.0 – 16.0	12.0 – 20.0	12.0 – 18.0
	4.0	13.0	13.0	13.0
Young leaves length	1.0 – 2.5	2.5 – 4.0	2.5 – 4.0	3.0 – 4.0
	2.0	2.5	3.5	4.0

Table 5 Effects of treatments (control and *gossan* wastes with amendment applied at 30, 75 and 150 Mg/ha) on growth of roots and shoots of *C. ladanifer* 505 days after sowing (minimum – maximum; mean; *n* = 3).

		Amended treatments		
Fresh biomass weight (g)	**Control**	**30 Mg/ha**	**75 Mg/ha**	**150 Mg/ha**
Total roots	2.4 – 7.7	13.5 – 10.0	13.8 – 17.3	11.3 – 13.0
	4.2[b]	15.5[a]	15.5[a]	12.2[a]
Roots per plant	0.08 – 0.25	0.41 – 0.61	0.36 – 0.48	0.33 – 0.46
	0.15[b]	0.50[a]	0.44[a]	0.38[a]
Total shoots	5.2 – 7.6	28.5 – 40.8	40.0 – 53.5	40.3 – 45.8
	6.4[c]	34.5[b]	44.8[a]	42.6[ab]
Shoots per plant	0.18 – 0.30	0.98 – 1.32	1.07 – 1.49	1.09 – 1.49
	0.23[b]	1.10[a]	1.27[a]	1.31[a]

Values for each parameter followed by a different letter are significantly different ($p < 0.05$).

Concentration of chemical elements in Cistus ladanifer

Despite the variations in the available concentrations of elements in GW from different treatments (Fig. 1 and Table 2), no relationship was found between these and the concentrations of elements in

C. ladanifer (roots and shoots; Tables 6 and 7). In fact, the concentrations of elements in *C. ladanifer* growing in mines from Portuguese IPB can depend on several biological processes (*e.g.* uptake, accumulation in roots, translocation from roots to shoots and tolerance capacity) related to the species or even ecotype, rather than concentration of elements in the soil available fraction (Santos et al., 2012, 2014). In general, roots and shoots had higher concentrations of all elements than those in the available fraction of GW. Exceptions were observed for Al, As and Fe in plant shoots from the amended treatments with the highest application rate.

Table 6 Effects of treatments (control and *gossan* wastes with amendment applied at 30, 75 and 150 Mg/ha) on the concentration of elements in *C. ladanifer* roots at the end of experiment (505 days after sowing) (Mean ± SD; $n = 3$).

		Amended treatments		
	Control	**30 Mg/ha**	**75 Mg/ha**	**150 Mg/ha**
		Hazardous elements (mg/kg)		
Al	$1.91 \times 10^3 \pm 529^{a}$	$1.55 \times 10^3 \pm 335^{ab}$	$0.85 \times 10^3 \pm 60^{c}$	$1.03 \times 10^3 \pm 307^{bc}$
As	36.21 ± 10.19^{a}	49.43 ± 11.43^{a}	41.15 ± 16.69^{a}	47.10 ± 11.03^{a}
Pb	7.00 ± 2.16^{a}	6.00 ± 0.87^{a}	5.67 ± 1.04^{a}	5.83 ± 1.04^{a}
		Macronutrients (g/kg)		
Ca	4.25 ± 1.96^{a}	4.17 ± 0.38^{a}	5.04 ± 0.50^{a}	5.99 ± 0.45^{a}
K	1.61 ± 0.29^{a}	1.99 ± 0.23^{a}	1.62 ± 0.39^{a}	1.75 ± 0.26^{a}
Mg	0.35 ± 0.11^{b}	0.46 ± 0.03^{ab}	0.57 ± 0.18^{ab}	0.66 ± 0.10^{a}
N	7.74 ± 2.05^{a}	2.81 ± 1.15^{b}	3.15 ± 0.56^{b}	3.27 ± 1.10^{b}
		Micronutrients (mg/kg)		
Cu	138.63 ± 32.71^{a}	39.33 ± 4.54^{b}	29.33 ± 3.62^{b}	30.67 ± 4.51^{b}
Fe	$1.69 \times 10^3 \pm 580^{a}$	$2.52 \times 10^3 \pm 555^{a}$	$1.99 \times 10^3 \pm 828^{a}$	$1.01 \times 10^3 \pm 455^{a}$
Mn	74.63 ± 9.01^{b}	142.67 ± 44.96^{a}	129.83 ± 14.84^{a}	107.33 ± 24.97^{a}
Na	$1.27 \times 10^3 \pm 246^{a}$	$1.57 \times 10^3 \pm 192^{a}$	$1.27 \times 10^3 \pm 322^{a}$	$1.37 \times 10^3 \pm 205^{a}$
Ni	228.88 ± 246.40^{a}	310.67 ± 191.85^{a}	313.00 ± 321.78^{a}	350.17 ± 204.77^{a}
Zn	531.88 ± 59.53^{a}	271.33 ± 50.58^{b}	157.50 ± 61.78^{c}	160.00 ± 43.41^{c}

Values for each element followed by a different letter are significantly different ($p < 0.05$).

Concentrations of As, Ca, K, Pb, Fe, Na and Zn in roots and concentrations of K, Mg and N in shoots were similar, independently of treatment. However, plants from control had larger concentrations of Al, N, Cu and Zn in roots and of Al, As, Cu, Fe, Na, Pb and Zn in shoots than those from amended treatments (Tables 6 and 7). In addition, the values of As and Pb in shoots from control reached values considered phytotoxic for plants (Kabata-Pendias, 2011). Although the accumulation depended on the plant species and amendments applied, similar results (higher concentrations in

shoots from control than amended treatments) were also reported by Qu and de Varennes (2010) using different amendments and plant species in a soil developed on *gossan*.

In shoots from all treatments, the concentrations of micronutrients and Ca were within the range considered sufficient for various plant species but concentrations of N, K and Mg are not considered adequate for growth (Kabata-Pendias, 2011; de Varennes, 2003). However, the increase in the availability of N, K and Mg in the GW following amendment application did not correspond to larger concentrations in shoots suggesting that adequate levels of these elements for *C. ladanifer* may be lower than those reported in the literature for other species. In fact, in natural conditions, shoots and roots of *C. ladanifer* collected in several mines from Portuguese IPB showed a wide range of all elements concentrations (Alvarenga et al. 2004; Batista et al. 2007, 2009; Freitas et al. 2004; Santos et al., 2012, 2013b,2014).

Table 7 Effects of treatments (control and *gossan* wastes with amendment applied at 30, 75 and 150 Mg/ha) on the concentration of elements in *C. ladanifer* shoots at the end of the experiment (505 days after sowing) (Mean ± SD; $n = 3$).

		Amended treatments		
	Control	**30 Mg/ha**	**75 Mg/ha**	**150 Mg/ha**
		Hazardous elements (mg/kg)		
Al	324.50 ± 143.77^{a}	204.23 ± 58.73^{a}	112.02 ± 59.92^{b}	94.23 ± 14.11^{b}
As	9.93 ± 4.27^{a}	3.39 ± 1.43^{b}	1.82 ± 0.53^{b}	4.13 ± 0.54^{b}
Pb	122.00 ± 55.22^{a}	27.33 ± 12.77^{b}	9.67 ± 2.47^{b}	10.17 ± 2.75^{b}
		Macronutrients (g/kg)		
Ca	10.94± 1.23^{b}	10.45 ± 0.76^{b}	12.67 ± 0.67^{a}	8.05 ± 0.58^{c}
K	4.53 ± 0.15^{a}	5.13 ± 0.19^{a}	5.01 ± 0.49^{a}	4.72 ± 0.09^{a}
Mg	0.75 ± 0.09^{a}	0.77 ± 0.03^{a}	0.92 ± 0.01^{a}	0.95 ± 0.20^{a}
N	6.79 ± 2.63^{a}	3.81 ± 0.55^{a}	4.28 ± 1.78^{a}	5.59 ± 1.08^{a}
		Micronutrients (mg/kg)		
Cu	28.75 ± 10.94^{a}	10.00 ± 2.60^{b}	5.33 ± 0.58^{b}	5.50 ± 0.50^{b}
Fe	299.06 ± 131.27^{a}	277.75 ± 50.52^{a}	91.12 ± 16.99^{b}	93.50 ± 14.89^{b}
Mn	68.00 ± 6.67^{b}	109.67 ± 42.00^{a}	72.33 ± 16.06^{ab}	49.33 ± 11.59^{b}
Na	707.88 ± 184.30^{a}	502.33 ± 74.74^{b}	480.17 ± 13.04^{b}	481.17 ± 46.54^{b}
Ni	6.50 ± 0.41^{a}	5.67 ± 0.29^{b}	4.83 ± 0.58^{c}	5.17 ± 0.29^{bc}
Zn	94.25 ± 14.75^{a}	95.33 ± 13.48^{a}	76.00 ± 12.77^{b}	70.00 ± 2.65^{b}

Values for each element followed by a different letter are significantly different ($p < 0.05$).

For *C. ladanifer*, the accumulation pattern suggests the existence of tolerance strategies related to the low fertility of GW and phytotoxicity of hazardous elements. Plants from control had an intensive uptake of the elements (even the hazardous) and accumulation of nutrients in photosynthetic active organs, whereas plants from amended treatments restricted the translocation to shoots of hazardous elements such as As and Pb. In this way, amendments application is an advantage for the success of phytostabilization.

CONCLUSIONS

The *gossan* waste studied had limiting characteristic for Cistus ladanifer growth, namely high total concentrations of several elements (Al, As, Cu, Fe, K and Pb), low fertility and acid pH although only a small percentage of the elements was in bioavailable forms. Amendment application, especially at 75 and 150 Mg/ha, improved physical (structure) and chemical properties (fertility, pH, concentrations of nutrients in the available fraction) of the *gossan* waste and increased enzymatic activities related to nutrient cycling and microbial activity. In addition, plants from the amended treatments had lower concentrations of hazardous elements in shoots than those from the control, minimizing potential bioaccumulation in the food chain.

Although *C. ladanifer* is already present in the São Domingos mine, the improvement in *gossan* waste may stimulate its growth in situ, providing a better plant cover as a result of faster growth during the phytostabilization process.

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REFERENCES

Abreu, M.M., Magalhães, M.C.F., 2009. Phytostabilization of soils in mining areas. Case studies from Portugal. In: Aachen, L., Eichmann, P. (Eds.), Soil remediation. Nova Science Publishers, Inc. New York, pp. 297-344.

Abreu, M.M., Santos, E.S., Magalhães M.C.F., Nabais C. 2009. Arsenic containing phases in soils from São Domingos mining area and in non-contaminated soils from Pomarão and Serra do Caldeirão. Revista de Ciências Agrárias 31(1), 155-169.

Abreu, M.M., Batista, M.J., Magalhães, M.C.F., Matos, J.X., 2010a. Acid mine drainage in the Portuguese Iberian Pyrite Belt. In: Brock, C., Robinson, (Ed.), Mine drainage and related problems. Nova Science Publishers, Inc. New York, pp. 71-118.

Abreu, M.M. Santos, E.S., Ferreira, M.B., Magalhães, M.C.F., 2010b. Availability of Cu, Pb and Zn in Canal Caveira and São Domingos mine areas from Iberian Pyrite Belt. Revista de Ciências Agrárias 33(1), 358-366.

Activation Laboratories, 2014. 1H - Total Digestion - ICP, INAA,. Available in: www.actlabs.com/page.aspx?page=506&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes2010 (Accessed January 2014)

Adriano, D.C., 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability and risk of metals. New York, Springer-Verlag.

Adriano, D.C., Wenzel, W.W., Vangronsveld, J., Bolan, N.S., 2004.Role of assisted natural attenuation in environmental cleanup. Geoderma, 122, 121-142.

Alvarenga, P.M., Araújo, M.F., Silva, J.A.L., 2004. Elemental uptake and root-leaves transfer in *Cistus ladanifer* L. growing in a contaminated pyrite mining area (Aljustrel-Portugal). Water, Air and Soil Pollution 152, 81-96.

Alvarenga, P., Palma, P., Gonçalves, A.P., Baião, N., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008a. Assessment of chemical, biochemical and ecotoxicological aspects in a mine soil amended with sludge of either urban or industrial origin. Chemosphere 72, 1774-1781.

Alvarenga, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008b. Evaluation of composts and liming materials in the phytostabilization of a mine soil using perennial ryegrass. Science of the Total Environment 406, 43-56

Álvarez-Valero, A.M., Pérez-López, R., Matos, J., Capitán, M.A., Nieto, J.M., Sáez, R., Delgado, J., Caraballo, M., 2008. Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian Peninsula): evidence from a chemical and mineralogical characterization. Environmental Geology 55(8), 1797-1809.

Batista, M.J., Abreu, M.M., Serrano Pinto, M., 2007. Biogeochemistry in Neves Corvo mining region, Iberian Pyrite Belt, Portugal. Journal of Geochemical Exploration 92, 159-176.

Batista, M.J., Gonzalez-Fernandez, O., Abreu, M.M., Carvalho, L., 2009.Chemical elements variation in leaves with different development stages of *Cistus* plants from S. Domingos mine area, South Portugal. Proceedings VII Congresso Ibérico e X Congresso Nacional de Geoquímica, pp. 407-415. Available in: http://repositorio.lneg.pt/bitstream/10400.9/740/1/33810.pdf. (Accessed June 2013)

Burgos, P., Madejón, P., Cabrera, F., Madejón, E., 2010. By-products as amendment to improve biochemical properties of trace element contaminated soils: Effects in time. International Biodeterioration & Biodegradation 64, 481-488.

CCME - Canada Council of Ministers of the Environment, 2007. Canadian soil quality guidelines for the protection of environmental and human health: summary tables (Updated September, 2007). CCME, Winnipeg, Canada.

Chao, T.T., 1972. Selective dissolution of manganese oxides from soils and sediments with acidified hydroxylamine hydrochloride. Soil Science 36, 764-768.

Conesa H.M., Schulin, R., Nowack, B., 2007. A Laboratory study on revegetation and metal uptake in native plant species from neutral mine tailings. Water, Air and Soil Pollution 183, 201-212.

Corral, R., Pita, J.M., Pérez-García, F., 1990. Some aspects of seed germination in four species of *Cistus* L.. Seed Science and Technology 18, 321-325.

de Endredy, A.S., 1963. Estimation of free iron oxides in soils and clays by photolytic method.Clay Minerals Bulletin 9, 209-217.

de Varennes, A., 2003. Produtividade dos solos e do ambiente. Escolar Editora, Lisboa.

de Varennes, A., Cunha-Queda, C., Qu, G., 2010. Amendment of an acid mine soil with compost and polyacrylate polymers enhances enzymatic activities but may change the distribution of plant species. Water, Air and Soil Pollution 208(1-4), 91-100.

Eivazi, F., Tabatabai, M.A., 1977.Phosphatases in soils. Soil Biology and Biochemistry 9, 167-172.

Eivazi, F., Tabatabai, M.A., 1988.Glucosidases and galactosidases in soils. Soil Biology and Biochemistry 20, 601-606.

EPA – United States Environmental Protection Agency 2000. Introduction to Phytoremediation. EPA/600/R-99/107

Feng, M., Shan, X., Zhang, S., Wen, B., 2005.A comparasion of rizosphere-based method with DTPA, EDTA, CaC_{l2} and $NaNO_3$ extraction methods for prediction of bioavailability of metals in soil to barley. Environmental Pollution 137, 231-240.

Freitas, H., Prasad, M.N.V., Pratas, J., 2004. Plant community tolerant to trace elements growing on degraded soils of São Domingos mine in the south east of Portugal: environmental implications. Environment International 30, 65-72.

Gonzaga, M.I.S., Ma, L.Q., Pacheco, E.P., Santos, W.M., 2012. Predicting arsenic bioavailability to hyperaccumulator Pteris vittata in arsenic-contaminated soils. International Journal of Phytoremediation 14, 939-949.

Harris, R.W., 1992. Root-shoot ratios. Journal of Arboriculture 18(1), 39-42.

Kabata-Pendias, A., 2004. Soil-plant transfer of trace elements-an environmental issue. Geoderma 122, 43-149.

Kabata-Pendias, A., 2011 Trace elements in soils and plants. 4th edition.CRC Press. Taylor & Francis Group, Boca Raton.

Kandeler, E., Gerber, H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biology and Fertility of Soils 6, 68-72.

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments − A review. Waste Management 28, 215-225.

Macías, F., 2004. Recuperación de suelos degradados, reutilización de resíduos y secuestro de carbono. Una alternativa integral de mejora de la calidad ambiental. Recursos Rurais 1, 49-56.

Macías, F., Macías-García, F., Nieto, C., Verde, J.R., Pérez C., Bao, M., Camps-Arbestain, M., 2011. Gestión de residuos y cambio climático, in: Mosquera, M.E.L., Osés, M.J.S. (Eds.), Gestión de residuos orgánicos de uso agrícola. Servizo de Publicacións e Intercambio Científico de la Universidade de Santiago de Compostela, Santiago de Compostela, 226 p..

Matos, J.X., Martins, L.P., 2006. Reabilitação ambiental de áreas mineiras do sector português da Faixa Piritosa Ibérica: estado da arte e prespectivas futuras. Boletín Geológico y Minero 117, 289-304.

Mendez, M.O., Maier, R.M., 2008. Phytoremediation of mine tailings in temperate and arid environments. Reviews in Environmental Science and Biotechnology 7, 47-59.

Pérez-López, R., Álvarez-Valero, A.M., Nieto, J.M., Sáez, R., Matos, J.X., 2008. Use of sequential extraction procedure for assessing the environmental impact at regional scale of the São Domingos Mine (Iberian Pyrite Belt). Applied Geochemistry 23, 3452-3463.

Qu, G., de Varennes, A., 2010. Use of hydrophilic polymers from diapers to aid the establishment of *Spergularia purpurea* in a mine soil. Journal of Hazardous Materials 178, 956-962.

Santos, E.S., Abreu, M.M., Nabais, C., Saraiva, J.A., 2009. Trace elements and activity of antioxidative enzymes in *Cistus ladanifer* L. growing on an abandoned mine area.Ecotoxicology 18, 860-868.

Santos, E.S., Abreu, M.M., Nabais, C., Magalhães, M.C.F., 2012. Trace elements distribution in soils developed on *gossan* mine wastes and *Cistus ladanifer* L. tolerance and bioaccumulation. Journal of Geochemical Exploration 123, 45-51.

Santos, E.S., Abreu, M.M., de Varennes, A., Macías, F., Leitão, S., Cerejeira, M.J. 2013a. Evaluation of chemical parameters and ecotoxicity of a soil developed on *gossan* following application of polyacrylates and growth of *Spergularia purpurea*. Science of the Total Environment 461-462, 360-370.

Santos, E.S., Nabais, C., Abreu, M.M., 2013b. Adaptabilidade ecofisiológica de diferentes populações de Cistus ladanifer L. do sul de Portugal. STUDIA – Scientiæ Rerum Diffusio: Suplemento Temático IV Seminário Luso-brasileiro em Ciências do Ambiente e Empresariais, 6 pp

Santos, E.S., Abreu, M.M., Batista, M.J., Magalhães, M.C.F., Fernandes, E. 2014. Inter-population variation on the accumulation and translocation of potentially harmful chemical elements in *Cistus ladanifer* L. from Brancanes, Caveira, Chança, Lousal, Neves Corvo and São Domingos mines in the Portuguese Iberian Pyrite Belt. Journal of Soil and Sediments14, 758-772.

Tabatabai, M.A., 1994. Soil enzymes. In: Mickelson, S.H., Bigham, J.M. (Eds.), Methods of soil analysis, Part 2, pp. 775-833.

Tordoff, G.M., Baker, A.J.M., Willis, A.J., 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere 41, 219-228.

Tu, S., Ma, L., Luongo, T., 2004.Root exudates and arsenic accumulation in arsenic hyperaccumulating *Pteris vittata* and non-hyperaccumulating *Nephrolepis exaltata*. Plant and Soil 258, 9-19.

Wang, X., Liu, Y., Zeng, G., Chai, L., Xiao, X., Song, X., Min, Z., 2008. Pedological characteristics of Mn mine tailings and metal accumulation by native plants. Chemosphere 72(9), 1260-1266.

Yao, F.X., Macías, F., Santesteban, A., Virgel, S., Blanco, F., Jiang, X., Camps Arbestain, M., 2009. Influence of the acid buffering capacity of different types of Technosols on the chemistry of their leachates. Chemosphere 74, 250-258.

Zhang S., Li W, Shan X.Q., Lu A., Zhou P. (2005). Effects of low molecular weight organic anions on the release of arsenite and arsenate from a contaminated soil. Water, Air and Soil Pollution 167(1-4), 111-122.

10. COMBINED REHABILITATION OF GOSSAN AND SULFIDE-RICH WASTES BY PHYTOSTABILISATION WITH AUTOCHTHONES SPECIES USING TECHNOSOLS

ABSTRACT

This study evaluated the recovery efficiency of a system combining two mine wastes: *gossan* (GW) and sulfide-rich wastes (SW). Technosols were made by the incorporation of amendment mixtures, at 30 and 60 g/kg, containing agro-industrial residues and GW. These Technosols (or only GW – control) overlie the sulfhide-rich wastes, which were isolated from air/water by an alkaline barrier. In the Technosols or GW, autochthones plants were developed (phytostabilisation process). Two microcosm assays, under controlled conditions, were carried out where *Cistus ladanifer* or *Lavandula pedunculata* were sown and grown for one and three years, respectively.

Technosols improved the structure, water-holding capacity and pH as well as increased the nutrients concentrations in the available fraction of the GW allowing the germination and good development of both species. No visual symptoms of deficiency/toxicity were observed although in some cases shoots had reached phytotoxic (As or Mn) or deficient (Cu) concentrations. Shoots elements concentrations were below the potentially hazardous level for domestic animals intake, and plants behaviours were adequate to their use in phytostabilisation (high uptake of elements, storage of hazardous elements in roots, non-accumulators of hazardous elements). Liming components of the barrier stabilised the SW reducing their oxidation and the ascension of the acid solutions and elements. Technosols were essential for the implementation of the phytostabilisation process in GW. The system of combined rehabilitation of GW and SW was efficient and sustainable for long-term.

KEYWORDS Alkaline barrier • *Cistus ladanifer* • *Lavandula pedunculata* • Organic/inorganic wastes • São Domingos mine

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INTRODUCTION

In the past, the mining activity in the Iberian Pyrite Belt (IPB) was developed without controlled rules for minimization of environmental or health risks. São Domingos (SE of Portugal, IPB) is a medium-sized mine that was exploited from the pre-roman period until 1960, both in the *gossan* and volcanogenic massive sulfides ore deposits (Matos et al., 2006; Quental et al., 2002). As a result of the different periods and techniques of exploration, between 27 and 32 Tg of different wastes were disposed over a large area affecting around 3.2 Mm^2 of land (Álvarez-Valero et al., 2008; Matos and Martins, 2006; Pérez-López et al., 2008; Quental et al., 2002). Each mine waste has different risks associated to its mass/volume, chemical composition and potential generation of acid mine drainage (Álvarez-Valero et al., 2008; Pérez-López et al., 2008). Taken into account the mobility of some elements and the mass/volume of *gossan* (4.1 Tg/1.6 Mm^3) and brittle pyrite (0.06 Tg/0.2 Mm^3), these materials represent the fourth and fifth, respectively, most hazardous mine wastes in the São Domingos mine area (Pérez-López et al., 2008).

São Domingos, as well as other mines from IPB, have an additional environmental problem because some of their mine wastes are composed of reactive minerals, especially sulfides, which generate acid drainage during all year (Abreu et al., 2010; Álvarez-Valero et al., 2008; Matos and Martins, 2006; Pérez-López et al., 2008; Quental et al., 2002; Sánchez-España, 2008).

Among the *in situ* technologies and comparing with the engineering techniques, phytostabilisation is considered the most suitable for the rehabilitation of different mine wastes or soils developed on mine wastes with multielemental contamination (Abreu and Magalhães, 2009; EPA, 2000; Monterroso et al., 1998), namely in arid and semiarid conditions (Mendes and Maier, 2008). Several environmental and economic advantages of this technology are reported by the same authors.

Some autochthones plant species are tolerant to the mining conditions (*e.g.* high total concentrations of trace elements, low pH and fertility and poor structure and water holding capacity) naturally colonizing mining areas from IPB, as for instance São Domingos mine (*e.g.* several species of *Cistus*, *Lavandula* and *Erica*) (Abreu et al., 2008, 2012a, 2012b; de la Fuente et al., 2010; Freitas et al., 2004a, 2004b; Pérez-López et al., 2014; Santos et al., 2012, 2014a). However, the sowing and establishment of the seedlings directly into tailings and/or soils developed on mine wastes can be very difficult or almost impossible, especially in Mediterranean climate (Tordoff et al., 2000). Besides, the slow growth rate of the plants can also limit the success of the phytostabilisation process.

Application of amendments can improve the establishment and development of these plants. In fact, although *Cistus ladanifer* is already present in the São Domingos mine, the improvement of the *gossan* wastes characteristics (*e.g.* structure, water-holding capacity, pH and nutrients concentrations in the available fraction of the materials) can stimulate its growth (Santos et al., 2014b) *in situ* providing a faster and better plant cover during the phytostabilization process.

In general, the amendments improve essential characteristics of the substrata (*e.g.* structure, fertility and water-holding capacity) for growth of plants and other organisms, and can immobilize potentially hazardous elements by several chemical processes (Adriano et al., 2004; Kumpiene et al., 2008; Tordoff et al., 2000; Wong, 2003). The use of amendments together with the plants contributes

to the occurrence of natural attenuation processes, increasing microbial biomass and fertility and appearance of spontaneous vegetation (Clemente et al., 2006), contributing to the carbon sequestration. However, some effects of the amendments may not last long (Macías, 2004; Macías et al., 2011) being necessary repetitive applications (Pérez-de-Mora et al., 2011). In addition, amendments mixtures should be adjusted to the physical and chemical conditions of each mine waste in order to produce a specific Technosol that promotes and keep the biogeochemical processes and reduces the availability of the potentially hazardous elements (Macías, 2004; Macías et al., 2011). In fact, the efficiency of the Technosols in the rehabilitation process of degraded/contaminated areas is confirmed in several in micro- and meso-cosmos studies under controlled conditions (Arbestainet al., 2008; Santos et al., 2014b, 2014c, 2015; Yao et al., 2009) as well as in the field (Bolaños et al., 2015; Macías-García et al., 2009).

Previous studies (Santos et al., 2014b, 2014c, 2015) demonstrated that although elements concentrations (*e.g*. As, Fe, K, Na, Pb and SO_4) and pH in leachates are significantly improved in the Technosols composed of sulfide-rich materials and organic/inorganic wastes, this methodology was not enough to reduce the sulfides oxidation or buffer leachates acidity. Nonetheless, the use of a Technosol obtained from *gossan* wastes and organic/inorganic wastes can be a promising approach to isolate sulfide-rich wastes from air allowing plant establishment and reduction of elements leaching from sulfide wastes (Santos et al., 2015). Indeed, in some situations a combined approach involving a covering material, which is sown with tolerant plants can have advantages rather any single conventional approach (Tordoff et al., 2000).

The main aim of this study was to demonstrate the efficiency of a system, which combined the rehabilitation of *gossan* wastes, by the conception of Technosols with agro-industrial wastes and development of autochthones plants (phytostabilisation process), and sulfide-rich wastes by its natural isolation from air/water. For this, the study aimed: i) to demonstrate the efficiency of a alkaline barrier (limestone wastes and biomass ashes) in the reduction of the upward flow of the acid solution rich in potentially hazardous elements from SW and decreasing of the leaching; ii) to evaluate the improvement of the chemical characteristics of *gossan* wastes by its inclusion in Technosols; and iii) to assess the development and accumulation of the elements (potentially hazardous elements and nutrients) in *Lavandula pedunculata* and *Cistus ladanifer*, at mid-term, during a phytostabilisation process with Technosols composed of *gossan* and organic/inorganic wastes

MATERIALS AND METHODS

Experimental set-up

Two mining wastes from São Domingos, which are mainly composed of *gossan* materials (hereinafter named as *gossan* wastes − GW) and crushed pyrite and smelting ashes (herein after named as sulfide-rich wastes − SW) were selected due to their large amounts of potentially hazardous elements, potential generation of acid mine drainage, wide distribution in the mine area, and, consequently, their environmental risk (Álvarez-Valero et al., 2008; Pérez-López et al., 2008; Quental et al., 2002). Moreover, it was also taken into account the potential of some of these mine wastes

(GW) to support the development of autochthones vegetation (Qu and de Varennes, 2010; Santos et al., 2013a, 2014b, 2015).

Composite samples of those mine wastes and seeds of *Lavandula pedunculata* (Mill.) Cav. and *Cistus ladanifer* L. were collected in the São Domingos mining area. Sampling areas of GW and SW were located south-west of the open pit, being the SW located near the former leaching tanks. The SW are bare and exposed to severe water erosion while GW are naturally but sparsely colonized mainly by *Erica australis* L..

The microcosm assay (*n* = 8 per treatment) were assembled in the following order (from bottom to top, Fig. 1): SW (total fraction at 50 % of water-holding capacity), limestone, biomass ashes and GW or Technosols (at 70 % of the water-holding capacity). The Technosols (Table 1) were composed of GW (fraction < 10 mm) and different amendment mixtures containing organic/inorganic wastes (equal proportion of each residue) from agro-industry: plant remains + strawberry substrate at 2:3 *m*/*m* – AgW; rockwool used for strawberry crops – RW; and residues from small distilleries − *Arbutus unedo* L. or *Ceratonia siliqua* L. fruit spirits distillation – AW, CW, respectively. The amendment mixtures and application rates used (Table 1) were based on the results of Santos et al. (2014b, 2015). The GW and amendment mixtures were mixed manually to create the Technosols. Limestone (L; Ø of 2−3 cm) and biomass ashes (BA) were used in order to create an alkaline barrier between SW and GW/Technosols.

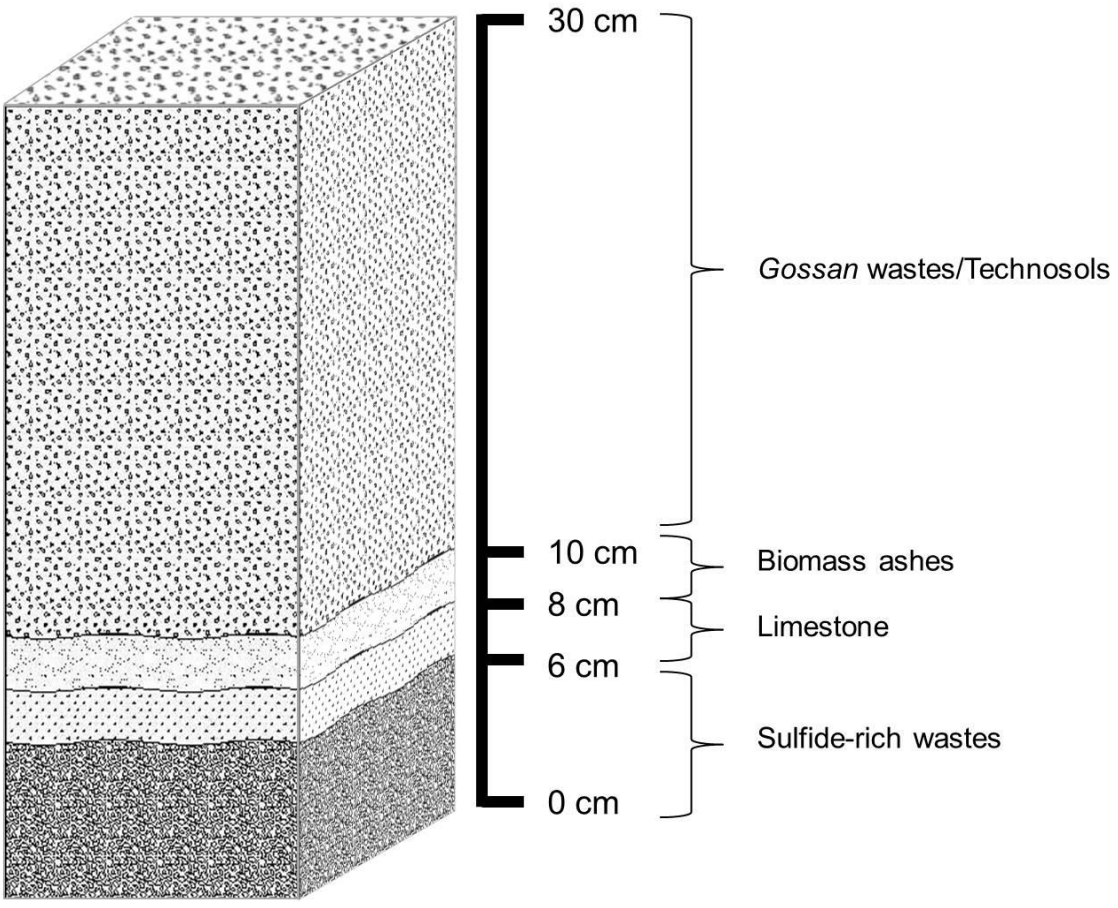

Fig. 1 Scheme of the combined rehabilitation system.

Table 1 Composition of the Technosols used in the assays.

Technosol	Composition	Rate of application of the amendment mixture (g/kg of mine waste) (designation)
TA	GW + (AgW + AW + RW)	30 (TA30), 60 (TA60)
TB	GW + (AgW + CW + RW)	30 (TB30); 60 (TB60)

GW: *gossan* wastes; AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops

All organic and inorganic wastes were used due to their physical and chemical characteristics (Table 2) and easy obtaining at very low cost (only associated to their transport) and large quantity, and location near the mine. Moreover, these wastes used in the Technosols are usually deposited in various locations without any treatment or legal permission and valorisation.

After one month of incubation of the GW and Technosols, *L. pedunculata* was sown (0.5 g seeds per pot) in half of the pots from each treatment, while in the other pots was sown pre-treated seeds of *C. ladanifer* (0.5 g seeds per pot; heating at 100 °C for 30 min; Corral et al., 1990). All pots were kept at 70 % of the maximum water-holding capacity under controlled conditions in a greenhouse for one and three years, for the assays with *L. pedunculata* and *C. ladanifer* respectively. Plants were thinned to 35, 20, 5 and 3 plants per pot after 40, 60, 120 and 180 days after sowing, respectively, to minimize plant competition.

Experimental monitoring and sample analyses

Initial mine wastes and organic/inorganic wastes were analysed for: pH and electrical conductivity (EC) in water suspension (1:2.5 *m*/*V*), organic C (Ströhlein method), extractable P and K (Egner-Riehm method), total N (Kjeldahl method) and total multielemental concentrations by ICP and INAA after acid digestion with four acids ($HClO_4$ + HNO_3 + HCl + HF; Activation Laboratories, 2014). For SW, biomass ashes and limestone, total C was analysed by combustion with a Leco analyser.

At the beginning (one month of incubation) and end of assays (after one and three years of *L. pedunculata* and *C. ladanifer* growth, respectively), composite samples of the GW and Technosols from each pot and corresponding to rhizosphere system were collected. A part of these samples were kept fresh (4 ºC) for determination of multielemental concentration in the available fraction, while the remaining subsamples were air-dried for classical characterization according methodologies referred above. To determine the available fraction, elements were extracted from GW and Technosols (fraction < 2 mm) by the rhizosphere-based method, which uses an aqueous solution at 10 mM mixture of organic acids (acetic acid + lactic acid + citric acid + malic acid + formic acid; Feng et al., 2005). Also, at the end of the experiment, materials from all the treatments were also collected, in each 5 cm of depth, and air-dried to determinate the total concentration of elements as to the initial materials.

At the end of the experiment, roots and shoots (composed of leaves and twigs) of both plant species were collected. Plant samples were washed with tap water and then with distilled water. After washing, the roots were sonicated in distilled water for 30 min. Plant samples were dried (40 ºC),

homogenised and finely ground. Elements (except N) were extracted from plant and certified reference samples with ultrapure concentrated nitric acid (69 %) under pressure in a microwave digester during 2 min at 85 ºC + 5 min at 145 ºC + 5 min at 200 ºC + 18 min at 200 ºC. Total concentration of N was analysed by combustion with a Leco analyser.

The aqueous solutions corresponding to the available fraction of GW and Technosols, and plant extracts were stored at –18 ºC until analysed for Al, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn by flame atomic absorption spectrometry and As by graphite furnace atomic absorption spectrometry. In plant extracts was still analyse P by visible spectrophotometry using the molybdenum blue method (USEPA, 1979).

Data analysis

Data were analysed by a one way ANOVA and the Duncan test ($p < 0.05$) used to separate means, using the statistical programme SPSS v18.0 for Windows. For statistical purposes, the results below the detection limit were assumed as half of the detection limit. Bivariate Pearson correlations were used to correlate GW or Technosols and plant characteristics ($r > 0.90$). Quality control of the analyses was made by analytical replicate samples, use of certified standards solutions, reference bush branches and leaves samples (NCSDC73348) and blanks. The accuracy control of the total concentration of the elements in GW and organic/inorganic wastes is guaranteed by the standards at the international accredited laboratory (Actlabs, ISO/IEC 17025; Activation Laboratories).

Three coefficients were calculated to evaluate the potential of both species for phytostabilisation with Technosols (Abreu et al., 2012a; Perelman, 1966): Soil-plant transfer coefficient (TransferC = [total shoots element]/[total soil element]), translocation coefficient (TranslC = [total shoots element]/[total roots element]), biological absorption coefficient (BAC = [total roots element]/[available soil element]). Soil-plant transfer coefficient indicates the accumulation behaviour of an element by plant (accumulator plants: TransferC > 1; non-accumulator/excluder: TransferC < 1). The translocation of an element from roots to shoots is evaluated by the translocation coefficient, while uptake capacity was assessed by biological absorption coefficient. Perelman (1966) classified the intensity of absorption by the roots in five groups: Intensive (10 < BAC < 100); Strong (1 < BAC < 10); Intermediate (0.1 < BAC < 1); Weak (0.01 < BAC < 0.1); Very weak (0.001 < BAC < 0.01).

RESULTS AND DISCUSSION

Characterisation of the initial materials

The chemical characteristics of the initial materials used in the microcosm assays are given in Table 2. *Gossan* wastes had acid pH, small EC and poor fertility characterised by very low concentrations of organic C and NPK. This material had great total concentrations of several hazardous elements, especially Al, As and Pb, and could be considered contaminated with As, Cu and Pb for commercial and industrial uses (CCME, 2007).

Table 2 Chemical characteristics of the *gossan* and the sulfide wastes from São Domingos mine area and organic/inorganic wastes used to make Technosols (min – max or mean value).

	GW	SW	AgW	AW	CW	RW	BA	L
pH (H_2O)	3.4 – 3.5	1.9 – 2.0	6.6 – 7.2	4.9	6.1	7.1	8.8	9.1
EC (mS/cm)	0.2 – 0.4	9.9 – 10.1	0.9 – 3.5	1.7	0.2	3.5	12.7	0.2
$C_{Organic}$ or C_{total}* (g/kg)	3.9 – 5.1	135.7 – 184.3*	286.1	442.6	436.7	111.0*	56.0*	122.0*
N_{Total} (g/kg)	0.1 – 0.2	1.7 – 2.2	9.7	8.9	11.1	9.8	0.3	—
$P_{Extractable}$ (g/kg)	< 0.02	< 0.02	0.3 – 3.1	0.2	0.1	7.9	—	—
$K_{Extractable}$ (g/kg)	0.3 – 0.7	0.2 – 0.3	0.2 – 6.6	3.6	9.1	0.7	—	—
				Total concentrations (g/kg)				
Al	19.9 – 21.5	41.2 – 43.3	4.06	1.37	0.6×10^{-3}	53.4	30.9	1.0
As	8.68 – 11.00	0.97 – 1.13	2.3×10^{-3}	1.0×10^{-3}	0.8×10^{-3}	$< 0.5 \times 10^{-3}$	0.03	29.4×10^{-3}
Ca	0.2 – 0.3	6.5 – 6.9	22.56	10.30	11.02	139.0	159.0	344
Cu	0.27 – 0.34	1.92 – 2.01	44.6×10^{-3}	0.12	9.4×10^{-3}	0.08	0.22	6×10^{-3}
Fe	210 – 255	86.1 – 96.1	2.96	3.30	0.99	39.90	19.8	1.7
K	3.2 – 3.3	10.6 – 11.3	1.495	> 1.32	0.96	6.80	25.4	0.4
Mg	0.3 – 0.4	1.5 – 1.6	2.34	1.13	> 9.63	42.10	14.7	2.5
Mn	0.04 – 0.06	0.11 – 0.12	0.25	51.2×10^{-3}	43.1×10^{-3}	1.85	2.88	68×10^{-3}
Na	0.7 – 0.8	3.6 – 3.9	0.31	0.40	0.40	13.10	5.5	0.1
Ni	0.01 – 0.02	0.05 – 0.06	5.9×10^{-3}	6.5×10^{-3}	1.9×10^{-3}	0.05	0.11	1×10^{-3}
Pb	31.6 – 34.5	8.36 – 9.28	5.0×10^{-3}	3.1×10^{-3}	0.9×10^{-3}	$< 3 \times 10^{-3}$	0.10	5×10^{-3}
S	9.0 – 9.1	60.0 – 67.5	—	—	—	2.60	11.6	0.2
V	0.06 – 0.07	0.15 – 0.16	9.0×10^{-3}	5.5×10^{-3}	1.4×10^{-3}	0.13	0.08	$< 2 \times 10^{-3}$
Zn	0.10 – 0.12	0.89 – 0.94	75.6×10^{-3}	27.3×10^{-3}	18.5×10^{-3}	0.20	0.25	5×10^{-3}

*Total C concentration; EC: electrical conductivity; GW: *gossan* wastes; SW: sulfide-rich wastes; AgW: Agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops; BA: biomass ash; L: limestone

Total concentrations of As, Cu, Fe, Mn, Ni, Pb, S, V and Zn were within the range of those reported by Álvarez-Valero et al. (2008) and Pérez-López et al. (2008) to the same type of materials and also from São Domingos. Nonetheless, GW used in this study presented worse chemical characteristics (total concentrations of several hazardous elements, especially As and Pb, pH and fertility) than similar materials from other studies (de Varennes et al., 2010; Qu and de Varennes, 2010; Santos et al., 2012, 2013a, 2014b, 2015) and also collected in the same mine area.

Sulfide-rich wastes had very acidic pH values as well as poor fertility and large EC. This mine waste also presented high total concentrations of several hazardous elements which were in the same range as those reported by several authors (Álvarez-Valero et al., 2008; Pérez-López et al., 2008; Santos et al., 2014c, 2015). With the exception of As and Pb, concentrations of the potentially hazardous elements in SW were higher than those in GW.

All the organic and inorganic wastes (AGW, AW, CW and RW) used in the amendment mixtures had chemical characteristics considered favourable and safe for land application and rehabilitation of GW. Moreover, chemical characteristics of L and BA reflected great potential to provide a natural and alkaline barrier layer between the GW or Technosols and the SW, due to their buffer capacity.

Chemical characterisation of the Technosols from microcosm assays

The chemical characteristics of the materials from GW and Technosols in the two assays, with *L. pedunculata* and *C. ladanifer* growth, are given in Table 3 and Figures 2 and 3. In general, characteristics of the GW were improved with the amendments application (Santos et al., 2014b) even during longer periods of plant growth (three years of plant growth). Comparing Technosols, significant differences were obtained in some characteristics between application rates of the amendments but not concerning to amendment mixture.

Even after only one month of incubation (beginning of the assay), it was observed an improvement of the Technosols structure, especially due to the incorporation of RW, which it remained until the end of the assays (data not shown). The incorporation of organic matter in the Technosols can also promote a favourable aggregation of particles and formation of granular structure (Zanuzzi et al., 2009). Additionally, the organic matter of the amendments from Technosols is oxidized originating weak organic acids with some buffering capacity what can explain the significant increase of pH of materials (Table 3). As expected, they also increased EC, due to the increase of nutrient amounts in the available fraction, but these values were still small compared to the application of other amendments (*e.g.* sewage sludge, compost from the organic fraction of unsorted municipal solid waste; Alvarenga et al., 2008a,b; Clemente et al., 2006).

In general, the amendments, independently of application rate and mixture, improved the fertility from contents very low to medium-high (INIA–LQARS, 2000). Concentrations of organic C and extractable P and K in the Technosols were higher than in GW (Table 3). This increase of the organic matter content contributed to the improvement of the water-holding capacity. The highest extractable K concentrations in GW at the end of the both assays are related to biomass ashes which, during the sampling, were slightly collected with GW. Thus, these results should not be considered as a real

difference compared to Technosols. Still concerning to extractable K, the amendments provided significant amounts in the beginning of the assays but, during the growth of both species, this nutrient was uptake by the plants. Technosols also had higher total N concentrations than GW (control) at beginning and after one year of *L. pedunculata* growth (Table 3). However, after three years of *C. ladanifer* growth, the highest total N concentration was obtained only in TB60. Taking into account that plants only grown in GW during approximately 40 days and the concentrations of NPK in Technosols where grown *L. pedunculata*, the results suggest that these elements remain for a significant long period in the Technosols and that their decrease at the end of *C. ladanifer* assay was possibly due to their uptake by plants.

Comparing the variations of C/N over time, the ratios for the Technosols suggest that until one year the mineralization and decomposition of organic matter is being favoured. In fact, only after three years of plants growth was observed a significant increase of organic C compared to the beginning (beginning = end of *L. pedunculata* assay but beginning < end of *C. ladanifer* assay). In this period, C/N ratios were very high (> 50) indicating that the mineralization is being very slow.

The Technosols and growth of both species enhanced much more the fertility of materials, compared to the application of hydrophilic polyacrylate polymers and growth of *Spergularia purpurea* (Pearson) G. Don fil. in soils developed on *gossan* (Santos et al., 2013a), but not the pH, which attained ≈7. In soils developed on *gossan*, after application of compost and *Dactylis glomerata* L. growth, pH only reached the maximum value of 5.9 although initial pH of the materials was 4.8 (de Varennes et al., 2010). In Technosols with the same composition of TA30 and TA60 but using a different GW and growing *C. ladanifer* for 15 months, the pH values were lower and fertility were quite different (Santos et al., 2014b) compared to the results obtained in the present study. This variation can be related to the heterogeneity of the organic and inorganic wastes used in the amendment mixture and the long experimental period (three years) of this study. It should be noticed that the GW used in the present study had much more extreme characteristics than similar materials referenced above, consequently, the result can be considered better.

The elements concentrations in the available fraction of GW and Technosols where *L. pedunculata* and *C. ladanifer* were grown are given in Figures 1 and 2, respectively. Independently of treatment, assay and sampling, the available concentrations of Ni and V were below detection limit of apparatus (lesser than ≈1 and ≈10 mg/kg, respectively).

In all Technosols and samplings (Figs. 2 and 3), the concentration of hazardous elements (Al and As) and nutrients (Ca, Fe, K, Mg, Mn, Na and Zn) in the available fraction increased, while Cu and Pb were reduced to values below the detection limit of the apparatus compared to the GW (mg/kg; Cu − GW: 1.5−2.1, Technosols: < 1; Pb – GW: 10.0−15.6, Technosols: < 5 mg/kg). Similar tendency was obtained in amended GW and soils developed on GW whose total concentrations of some elements are quite different (de Varennes et al., 2010; Santos et al., 2013a; 2014b). With application of biosolids to copper mine tailings Santibáñez et al. (2010) found higher concentration of Zn in the pore-water, as well as an increase on Cu concentration. This last finding is not in agreement with the results obtained in the present study. In a soil from the area affected by the Aznalcóllar mine accident amended with different wastes (alperujo, biosolid compost and sugar beet lime), the concentrations of Cu, Mn and Zn

in the available fraction (extracted with $CaCl_2$) were, in general, reduced (Burgos et al., 2010), which can be a disadvantage to the development of plants and other organisms.

Table 3 Characteristics of the *gossan* wastes and Technosols collected at the beginning (after one month of incubation) and end of the assays (after one and three years of *Lavandula pedunculata* and *Cistus ladanifer* growth, respectively) (mean ± SD; n = 4).

		Technosols			
	GW	**TA30**	**TA60**	**TB30**	**TB60**
		Beginning for both assays			
pH (H_2O)	3.7 ± 0.03^{c}	5.8 ± 0.3^{a}	5.8 ± 0.2^{a}	5.0 ± 0.1^{b}	6.0 ± 0.1^{a}
EC (µS/cm)	137 ± 21^{b}	381 ± 117^{ab}	749 ± 413^{a}	432 ± 170^{ab}	620 ± 293^{a}
$C_{organic}$ (g/kg)	2.2 ± 0.2^{d}	8.5 ± 1.9^{b}	12.7 ± 2.0^{a}	6.2 ± 0.2^{c}	9.0 ± 1.7^{b}
N_{Total} (mg/kg)	125.8 ± 10.2^{d}	368.1 ± 47.9^{b}	489.7 ± 143.9^{a}	252.1 ± 61.4^{c}	430.6 ± 31.5^{ab}
$P_{Extractable}$ (mg/kg)	0.2 ± 0.2^{d}	42.5 ± 22.0^{c}	162.6 ± 84.1^{b}	35.9 ± 19.8^{c}	328.7 ± 21.5^{a}
$K_{Extractable}$ (mg/kg)	16.9 ± 0.5^{c}	85.9 ± 21.5^{bc}	159.8 ± 61.1^{b}	139.1 ± 20.8^{b}	308.6 ± 87.9^{a}
		End of the assay with Lavandula pedunculata			
pH (H_2O)	4.2 ± 0.2^{c}	6.0 ± 0.3^{a}	5.9 ± 0.2^{ab}	5.6 ± 0.2^{b}	6.0 ± 0.1^{a}
EC (µS/cm)	1003 ± 659^{a}	226 ± 147^{b}	468 ± 198^{b}	285 ± 156^{b}	335 ± 221^{b}
$C_{organic}$ (g/kg)	3.1 ± 1.3^{c}	7.5 ± 0.6^{b}	10.9 ± 1.1^{a}	6.8 ± 1.2^{b}	9.4 ± 1.0^{a}
N_{Total} (mg/kg)	118.6 ± 10.8^{d}	368.7 ± 56.5^{b}	514.1 ± 65.3^{a}	287.7 ± 34.8^{c}	395.5 ± 24.3^{b}
$P_{Extractable}$ (mg/kg)	3.0 ± 3.2^{c}	149.3 ± 16.0^{b}	525.9 ± 217.4^{a}	147.9 ± 120.2^{b}	515.8 ± 166.2^{a}
$K_{Extractable}$ (mg/kg)	513.2 ± 253.9^{a}	95.5 ± 54.6^{b}	202.6 ± 43.4^{b}	119.3 ± 10.6^{b}	219.8 ± 87.5^{b}
		End of the assay with Cistus ladanifer			
pH (H_2O)	4.0 ± 0.2^{b}	5.8 ± 0.3^{a}	5.9 ± 0.1^{a}	5.8 ± 0.2^{a}	5.7 ± 0.2^{a}
EC (µS/cm)	1090 ± 369^{a}	199 ± 144^{b}	268 ± 70^{b}	471 ± 364^{b}	102 ± 23^{b}
$C_{organic}$ (g/kg)	5.2 ± 0.5^{c}	15.2 ± 1.1^{b}	26.2 ± 4.2^{a}	15.9 ± 2.4^{b}	22.6 ± 4.6^{a}
N_{Total} (mg/kg)	341.2 ± 126.9^{ab}	388.2 ± 75.5^{ab}	204.4 ± 151.2^{b}	234.5 ± 104.2^{b}	444.1 ± 93.1^{a}
$P_{Extractable}$ (mg/kg)	0.9 ± 0.5^{c}	68.1 ± 22.3^{b}	176.8 ± 100.5^{a}	79.6 ± 25.0^{ab}	169.8 ± 88.2^{a}
$K_{Extractable}$ (mg/kg)	474.3 ± 41.7^{a}	76.8 ± 12.2^{b}	79.9 ± 13.6^{b}	63.9 ± 16.5^{b}	79.9 ± 24.0^{b}

EC: electrical conductivity; GW: *gossan* wastes; TA30 and TA60: Technosols containing GW and AgW+AW+RW at 12 and 30 g/kg; TB30 and TB60: Technosols containing GW and AgW+CW+RW at 12 and 30 g/kg. AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops
Values for each parameter and sampling area followed by a different letter are significantly different (p < 0.05).

The high concentrations of Al in the available fraction are related to the incorporation of rockwool in the amendments mixture, which had Al in its composition, while the nutrients come particularly from

the remaining nutrient solution incorporated in the rockwool used to grow strawberry (Table 2). The majority of the nutrients can also derived from organic matter, although statistically only moderate correlation was obtained ($0.66 < r < 0.80$, depending on element, sampling and assay).

The effect of organic matter in the mobilization or immobilization of the elements depends on their characteristics (Bolan et al., 2014). Santibáñez et al. (2010) attributed the high availability of elements in amended substrates to the increase of dissolved organic C amounts released from organic amendments decomposition. However, organic matter from Technosols can also have increased the cationic exchange capacity and promoted the formation of complexes with some cations (Adriano et al., 2004; Bolan et al., 2014; Kumpiene et al., 2008) justifying the reduction of Cu and Pb (GW: 1.5−2.1 mg Cu/kg and 10−15.6 mg Pb/kg depending on assay and sampling) of more than 15 % and 45 % respectively (although obtaining moderate correlations). Several authors (Alvarenga et al., 2008a, 2008b; Burgos et al., 2010) ascribed the decrease on the Cu and/or Pb concentrations in the available fraction of acid and metal-contaminated soils to the application of organic amendments (*e.g.* sewage sludge or biosolid compost) to these soils. Besides, pH can affect availability of Cu and Pb, however no strong relationship was obtained between pH and the available concentrations of these elements. This fact was also observed in other studies (Alvarenga et al., 2008a, 2008b; Burgos et al., 2010; Santos et al., 2013a).

The increase of As can be related to the inhibition of the formation of some solid phases with low solubility (*e.g.* metal arsenates) (Abreu et al., 2009; Santos et al., 2012). The increase of dissolved organic C in the rhizosphere due to exudation of low molecular weight organic acids by roots and/or organic wastes from amendment mixtures may also justify the increase of As availability in the Technosols (Bolan et al., 2014; Feng et al., 2005; Kumpiene et al., 2008; Tu et al., 2004). In fact, some root exudates or even specific low molecular-weight organic acids can release As from arsenates (Tu et al., 2004) and metal oxi-hydroxides (Zhang et al., 2005).

Comparing the effect of the amendments on the GW to make the Technosols, no significant differences were obtained between available concentrations of K, Mg, Mn and Na according on amendment mixture and application rate at beginning of the assays and the control, whereas the same occurred for Al, K, Mg, Na and Zn at the end of the two assays. For the other elements and in all sampling periods, the application rate altered the concentrations of the elements in the available fraction having, in general, the highest amounts with application of 60 g/kg of amendments. Exceptions were observed for Al, As, Fe and Zn in the beginning of the assay (after one month of incubation), where a combined effect can have occurred with the type and amount of the organic matter because there is no clear trend in the application rate and amendment mixture. It should be further highlighted the variation of the available concentrations of As and Fe among Technosols according to the species (and possibly duration of the assay). Thus after one year of *L. pedunculata* growth, Fe concentrations were similar among Technosols (108.2−134.8 mg/kg depending on treatment) while the highest As values were obtained in the Technosols at 60 g/kg of amendments (mg/kg − TA60: 6.1; TB60: 6.8; TA30: 4.5; TB30: 3.4). However, the opposite was verified after three years of *C. ladanifer* growth (mg/kg – As: 3.6−4.4, depending on treatment; Fe: TA60: 219.9; TB60: 201.6; TA30: 180.0; TB30: 177.3).

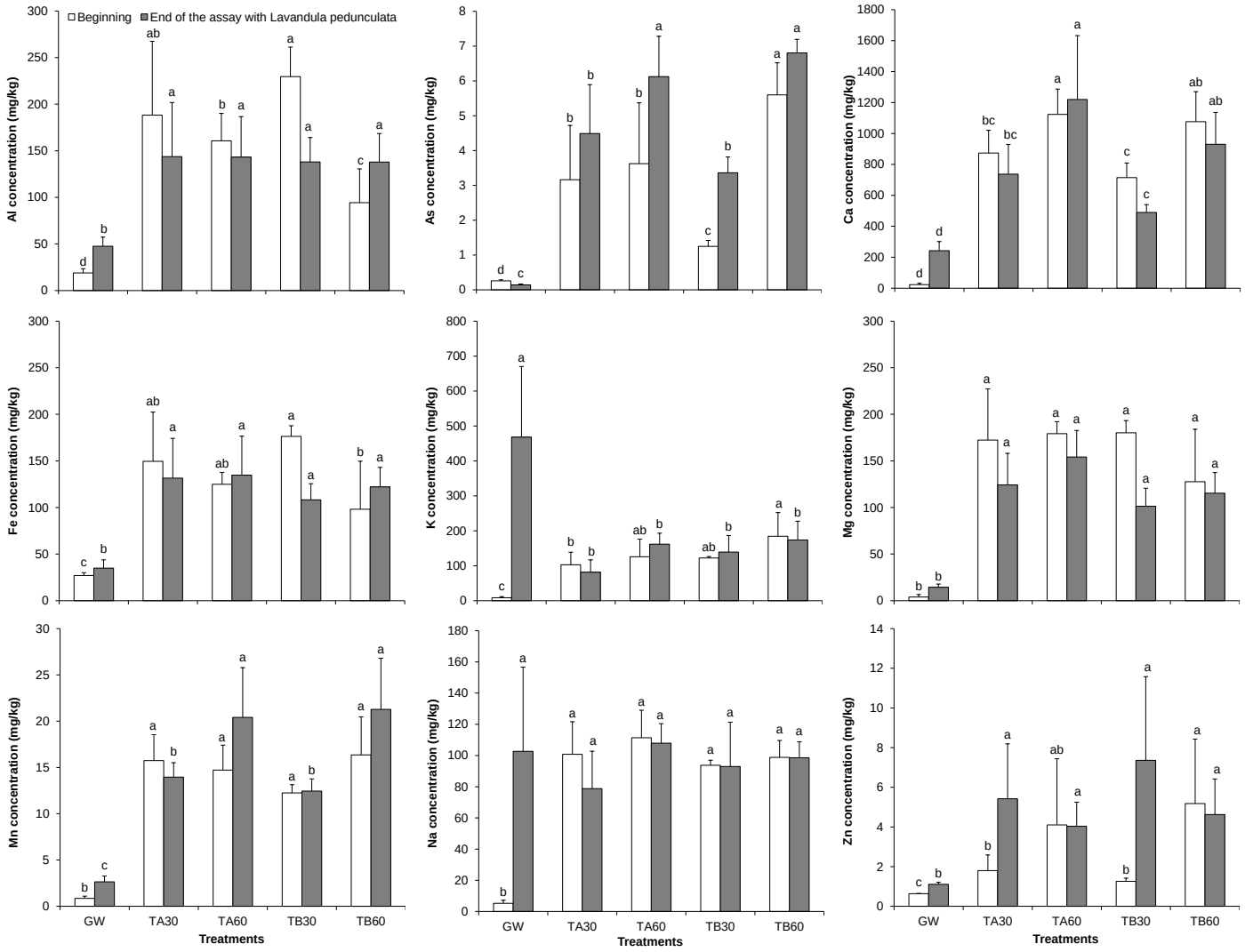

Fig. 2 Effects of treatments (*gossan* wastes and Technosols) on the concentration of the elements in the available fraction at the beginning (after one month of incubation) and end of the assay with *Lavandula pedunculata* (after one year of plants growth) (mean ± SD; n = 4). Values from same sampling period followed by a different letter are significantly different ($p < 0.05$).

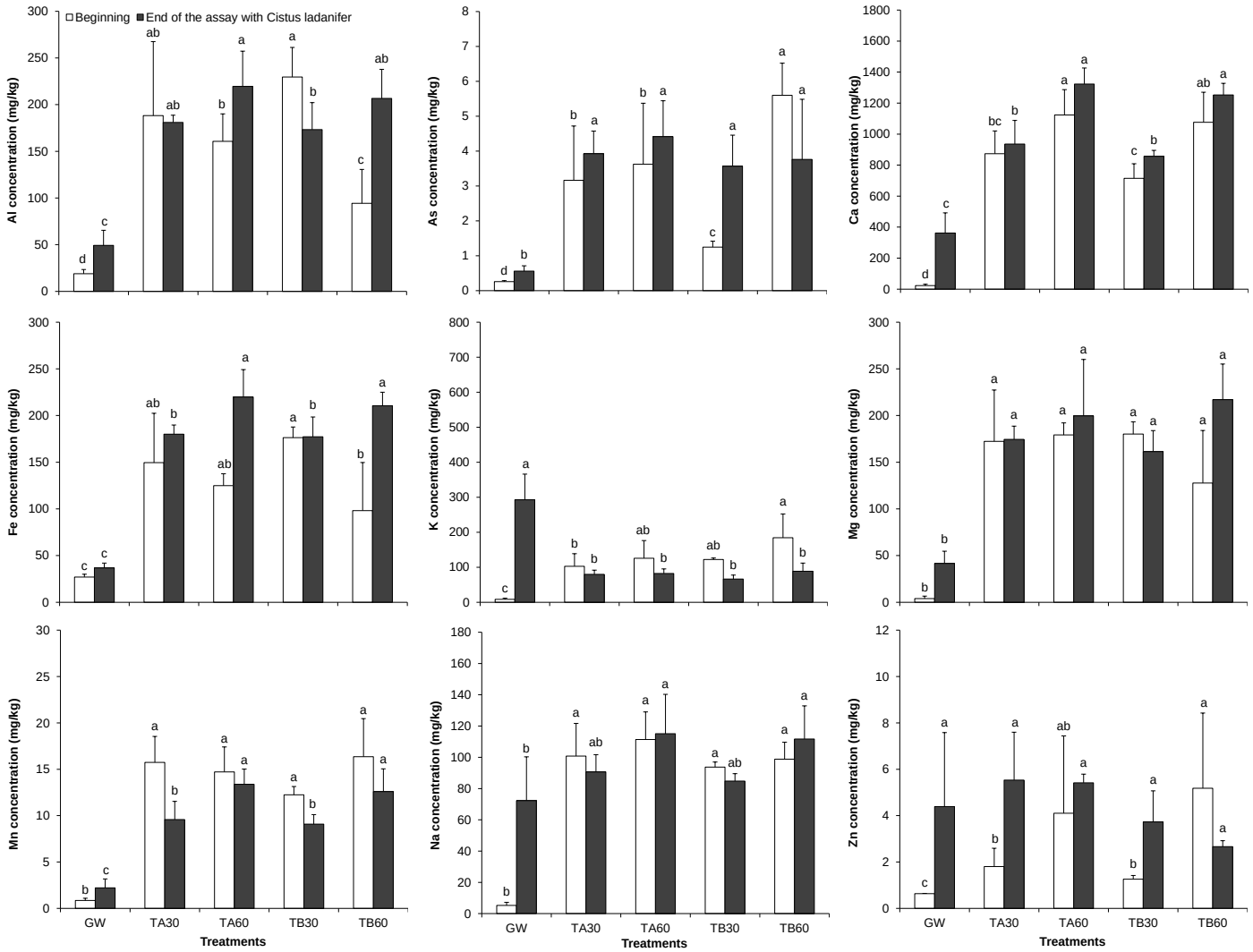

Fig. 3 Effects of treatments (*gossan* wastes and Technosols) on the concentration of the elements in the available fraction at the beginning (after one month of incubation) and end of the assay with *Cistus ladanifer* (after three years of plants growth) (mean ± SD; $n = 4$). Values from same sampling period followed by a different letter are significantly different ($p < 0.05$).

In general, concentrations of the elements in the available fraction increased over time in GW (control), possibly by the speed-up of the materials weathering due to irrigation. However in the Technosols, this tendency was only observed in some cases (*e.g.* Zn in both plants assays; Ca and Fe in *L. pedunculata* assay). In fact, the amendments provided sufficient nutrients amounts to support the plant growth (and all microbial community) and keep the stable concentrations over time. Concentrations of the potentially hazardous elements (Al and As) increased from the beginning till one year, but after that showed a tendency to be constant (except As in TA30 and TA60 and Al in T60B).

Although GW had large total concentrations of Al, As, and Pb, the amounts of these elements potentially available for the plants and microorganisms in the Technosols were lesser than 1 % of the total concentrations, which represent a small potential environmental risk. Nonetheless, the increase of organic C and nutrients availability and their permanence in the soil system during longer periods is an advantage for the rehabilitation process.

In general, no clear improvement of the chemical characteristics of Technosols was observed both with amendment mixture and/or application rate.

Germination and growth of Lavandula pedunculata and Cistus ladanifer

Germination of both plant species was stimulated in the Technosols, compared to GW (Table 4). Approximately 40 days after sowing, seedlings of *L. pedunculata* (≈4 cm height) and *C. ladanifer* (≈3 cm height) growing in GW and without visible symptoms of nutritional deficiency or phytotoxicity died. This fact is not in agreement with the results obtained by Santos et al. (2014b) in which *C. ladanifer* survived (with a very small development) for 505 days in a *gossan* waste also collected in São Domingos mine area but with better chemical characteristics than GW used in the present study. Nonetheless, in a mesocosm assay with soils developed on *gossan* materials and considerable quantity of host rocks (*i.e.* also with less extreme conditions than the GW studied) was observed germination of *C. ladanifer* in control but not of *Lavandula sampaioana* (Rozeira) Rivas Mart., T.E. Díaz & Fern. Gonz. But the mortality was also very high in both species (Santos et al., 2013a). The improvement of the structure and water holding capacity, due to the amendments in the Technosols, can explain the higher germination rate of both species in the present study. Moreover, these parameters of the materials combined with the increase of nutrients and organic C (Figs. 2 and 3, and Table 3) can justify the survival and great plant growth during all assays in the Technosols.

Table 4 Effects of the treatments (*gossan* wastes and Technosols) on germination (%) of *Lavandula pedunculata* and *Cistus ladanifer* (mean ± SD; *n* = 4).

		Technosols			
Species	**GW**	**TA30**	**TA60**	**TB30**	**TB60**
Lavandula pedunculata	3 ± 2^{b}	15 ± 9^{a}	18 ± 10^{a}	17 ± 9^{a}	16 ± 9^{a}
Cistus ladanifer	1 ± 1^{c}	6 ± 1^{b}	11 ± 5^{a}	6 ± 2^{b}	5 ± 3^{b}

GW: *gossan* wastes (control); TA30 and TA60: Technosol containing GW and AgW+AW+RW at 30 and 60 g/kg; TB30 and TB60: Technosol containing GW and AgW+CW+RW at 30 and 60 g/kg; AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops
Values for each species followed by a different letter are significantly different ($p < 0.05$)

Biomass ashes from bottom of the Technosols can also supply several nutrients to the plants (Ram and Masto, 2010; Su and Wong, 2003) ensuring their growth for long periods. The fertility of the materials is considered the parameter with major importance for the success of phytostabilisation, especially in the early stages of plant growth (Tordoff et al., 2000). However the success of phytostabilization of mine wastes, especially those located in areas with Mediterranean climate, depends on a combined effect of several characteristics (*e.g.* hydric stress).

The growth stimulation of different herbaceous (*e.g. D . glomerata, Lolium multiflorum* Lam and *Lolium perenne* L.) and shrubs (*e.g. C. ladanifer* and *Erica australis L.*) in microcosm assays under controlled conditions (Alvarenga et al., 2008a, 2008b; de Varennes et al., 2010; Qu and de Varennes, 2010; Santibáñez et al., 2008; Santos et al., 2013a, 2014b) as well as spontaneous vegetation in the field (Pérez-de-Mora et al., 2011) in contaminated soils/mine wastes after different amendments application is reported. None of the other characteristics of the Technosols (Figs. 2 and 3, and Table 3) seem to affect negatively the plants growth.

Although Technosols with high application rates of amendments had greater concentrations of organic C and nutrients (*e.g.* total N and extractable P; Table 3), no significant variation was observed in height and fresh shoots biomass of *L. pedunculata* (Table 5). Even for fresh roots biomass, it was only observed a slight variation according to application rate of the amendments in spite of plants from TB30 have reached the smallest values.

Table 5 Effects of the Technosols on growth of *Lavandula pedunculata* and *Cistus ladanifer* (mean ± SD; $n = 4$).

Plant parameters	Technosols			
	TA30	**TA60**	**TB30**	**TB60**
Lavandula pedunculata (after one year of growth)				
Plant height (cm)	43 ± 11^{b}	37 ± 7^{b}	34 ± 6^{b}	32 ± 3^{b}
Fresh roots weight (g)	21.4 ± 6.9^{bc}	25.9 ± 3.4^{b}	16.3 ± 4.1^{c}	22.9 ± 5.0^{bc}
Fresh shoots weight (g)	47.2 ± 21.1^{b}	52.2 ± 4.6^{b}	44.4 ± 11.2^{b}	54.5 ± 8.0^{b}
Cistus ladanifer (after three years of growth)				
Plant height (cm)	61 ± 10^{bcd}	78 ± 19^{b}	58 ± 13^{d}	75 ± 19^{bc}
Fresh roots weight (g)	23.0 ± 4.8^{b}	30.9 ± 10.0^{b}	23.6 ± 4.7^{b}	25.3 ± 3.2^{b}
Fresh shoots weight (g)	45.9 ± 7.5^{c}	76.4 ± 8.2^{b}	41.2 ± 4.9^{c}	67.9 ± 19.2^{b}

TA30 and TA60: Technosols containing GW and AgW+AW+RW at 30 and 60 g/kg; TB30 and TB60: Technosols containing GW and AgW+CW+RW at 30 and 60 g/kg; AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops
Values for each parameter and plant species followed by a different letter are significantly different ($p < 0.05$)

In the assay with *C. ladanifer*, the plant growth was during a longer period (three years) so, even being used big pots, the inner space of the pot can have limited the development of the radicular system. Nevertheless the height and, especially, fresh shoots biomass indicated greater plant development in Technosols with the highest application rate of amendments. This results are in agreement with those reported by Santos et al. (2014b) for 15 months of *C. ladanifer* growth in

another amended *gossan*, as well as with other plant species also growing in a soil developed on other *gossan* presenting different characteristics (de Varennes et al., 2010; Qu and de Varennes, 2010).

Multielementar composition of Lavandula pedunculata and Cistus ladanifer

Potentially hazardous elements

The behaviour of the two studied species in the absorption, translocation and accumulation of the potentially hazardous elements was similar. However, the concentrations of the elements in the roots ad shoots depended on species (Fig. 4). The uptake (evaluated by the biological absorption coefficient) was, mainly, strong for Al (*L. pedunculata*: 4−15; *C. ladanifer*: 0.3−6) and intensive for As and Pb (BAC_{As}: 7−97; BAC_{Pb}: 56−381, depending on species). After absorption, and, as a tolerance mechanism (Kabata-Pendias, 2011), these elements were stored in their roots (TranslC < 0.3).

No significant differences were obtained among the concentrations of Al in roots (849.3−1222.9 mg/kg) and As and Pb in shoots mg/kg; 5.6−10.7 and 17.4−27.2, respectively) of *L. pedunculata* from different Technosols. The highest concentrations of As and Pb in roots of this species were reached in TA30 (130.1 mg As/kg and 459.4 mg Pb/kg) which were the double of the values determined in the plants from the other Technosols (55.5−72.4 mg As/kg and 229.7−279.9 mg Pb/kg, depending on treatment). Slight variation of Al concentrations in shoots was observed, however *L. pedunculata* growing in Technosols with high amendments application rates showed a tendency to have higher values (mg/kg − TA60: 153.4; TB60: 178.2; TA30: 99.1; TB30: 112.0). In *C. ladanifer*, the concentrations of the studied potentially hazardous elements in roots and shoots were similar independently of the Technosol where they grow. Despite of the variations in the elements concentrations in the available fraction of the different Technosols (Figs. 2 and 3), no relationship was found between this soil characteristic and the concentrations of the potentially hazardous elements in roots or shoots of both species.

Phytotoxic concentrations of the elements in shoots were only reached and exceeded for As (5 mg/kg; Kabata-Pendias, 2011) for: *L. pedunculata* from all Technosols and *C. ladanifer* from Technosols containing residue from liquor distillation of *C. siliqua* fruit (TB30 and TB60). However, no visual symptoms of toxicity were observed in the two species. In *L.* pedunculata a possible synergism effect of Pb in shoots can explain these phytotoxic values of As (r = 0.99). Both species were non-accumulators of the studied hazardous elements.

In spite of the variability intra and inter-population of *C. ladanifer* growing in mine wastes and/or soils developed on mine wastes in natural conditions (Santos et al., 2012, 2014a and references therein), the plants obtained in this study, under controlled conditions, showed a similar behaviour on the translocation and accumulation, being the elements concentrations in the same range than the field. Regarding *L. pedunculata*, lower concentrations of As and Pb in shoots were also reported for plants growing in mining areas from Rio Tinto region (de la Fuente et al., 2010) and serpentine area from NE of Portugal (Freitas et al., 2004b).

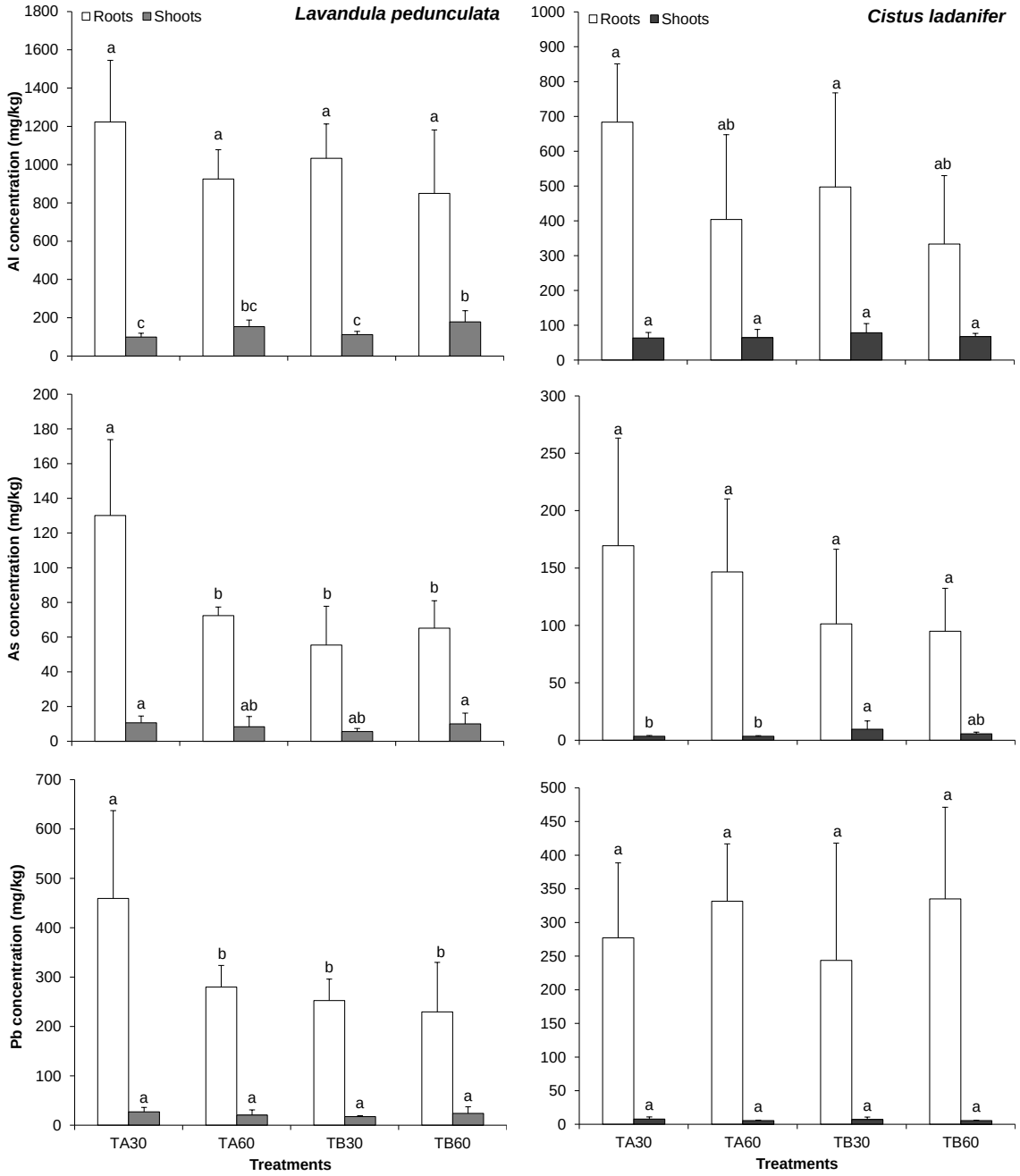

Fig. 4 Effects of the Technosols on the concentration of the potentially hazardous elements in the roots and shoots of *Lavandula pedunculata* and *Cistus ladanifer*. Values from same species, organ and element followed by a different letter are significantly different ($p < 0.05$).

Nutrients

In general, the plants uptake capacity of the nutrients, evaluated by the biological absorption coefficient, was strong and intensive but depended on the species having in some cases variability intra-treatment. The absorption of Ca, Ni and P was mainly strong in *L. pedunculata* (BAC: 3−27, depending on element and treatment) and the same uptake capacity for Ca, Fe, Mg, Mn, Na, Ni and P

(BAC: 1−32, depending on element and treatment) was observed in *C. ladanifer*. The other nutrients presented intensive uptake (*L. pedunculata*: Cu, Fe, Mg, Mn, Na and Zn; *C. ladanifer*: Cu and K).

Plant of both species showed some variability in the translocation behaviour of the nutrients even growing in same treatment, however a clear tendency can be indicated for each species (Figs. 5−8). Variation of the translocation behaviour of some elements can be observed in several species growing spontaneously in mining areas from IPB (Abreu et al., 2012a, 2012b; Pérez-López et al., 2014; Santos et al., 2012, 2014a). Both species translocated, mainly, from roots to shoots K, N and P (TranslC: 0.5−4.5 depending on element, species and treatment) while Cu, Fe, Na and Ni were stored in the roots. *Cistus ladanifer* still translocated Ca, Mg and Zn but *L. pedunculata* stored these nutrients in their roots (TranslC − *L. pedunculata*: 0.4−1.2; *C. ladanifer*: 1.0−13.4, depending on element and treatment). The opposite occurred for Mn being stored in roots of *C. ladanifer* (TranslC: 0.3−1.8, depending on treatment) but translocated in *L. pedunculata* (TranslC: 1.8−7.2, depending on treatment).

Concentrations of the nutrients in roots and shoots also varied with species (Figs. 5−8) being the combined effect of the uptake, accumulation in roots, translocation from roots to shoots, a tolerance capacity and, possibly, nutritional requirements. As for the available fraction of the elements (Figs. 2 and 3, and Table 3), no clear tendency was obtained among the plants from different Technosols. In fact, only in *L. pedunculata* shoots from Technosols containing 60 g/kg of amendments showed the highest Mg concentrations (mg/kg − TA60: 2.1; TB60: 1.9), compared to plants growing in a Technosol with half of the amendment dose (≈1.3 mg/kg). Moreover, independently of the Technosol, no significant differences were obtained among concentrations of Ca, Mn and Na in roots and shoots and Cu, Fe, N, P and Zn in shoots of *L. pedunculata*. The same was observed in *C. ladanifer* for Ca, Cu in shoots, Na and Ni in roots and K, Mg, N, P and Zn in roots and shoots. No relationship was obtained between nutrients concentrations in roots or shoots of the two species and the respective available fractions of the elements in the Technosols.

Although *C. ladanifer* had presented an effective uptake capacity of Cu and Mn, their storage in roots (or low translocation) can contribute, to some extent, to the deficient concentrations of these elements in the shoots. Thus, *C. ladanifer* shoots from all the Technosols presented deficient Cu concentrations (< 5 mg/kg; Kabata-Pendias, 2011) but, only shoots from Technosols containing *A. unedo* residue had deficiency of Mn (< 30 mg/kg; Adriano, 2001; Kabata-Pendias, 2011).The great translocation of Mn in *L. pedunculata* from all the Technosols and Zn in plants from the Technosols at 30 g/kg amendments application can explain the concentrations of these elements in the shoots, which are considered phytotoxic or above the normal/sufficient (> 400 mg Mn/kg and > 150 mg Zn/kg; Kabata-Pendias, 2011). None synergism or antagonism effect between elements was statistically obtained, allowing to explain these anomalous concentrations in shoots of both species. Moreover, in any case, the plants did not show visual symptoms of deficiency or toxicity indicated by several authors (Adriano, 2001; Kabata-Pendias, 2011). Concentrations of the remaining nutrients in the shoots of the two species reached values considered normal/sufficient and/or below the phytotoxicity for the majority of the plant species (Adriano, 2001; Kabata-Pendias, 2011). In *C. ladanifer* a possible synergism effect of As in shoots can occur with Fe (r = 0.91).

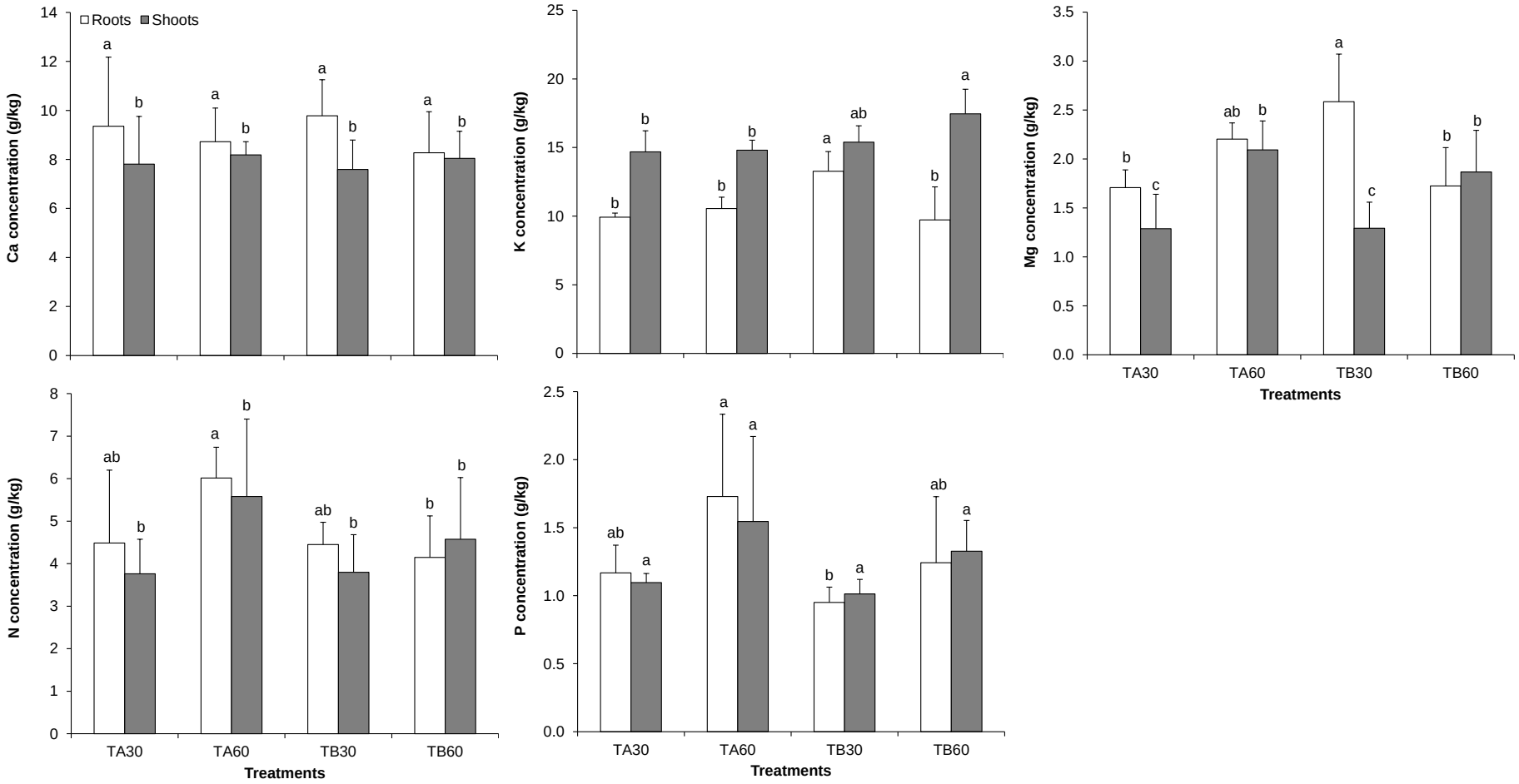

Fig. 5 Effects of the Technosols on the concentration of macronutrients in the roots and shoots of *Lavandula pedunculata*. Values from same organ and element followed by a different letter are significantly different ($p < 0.05$).

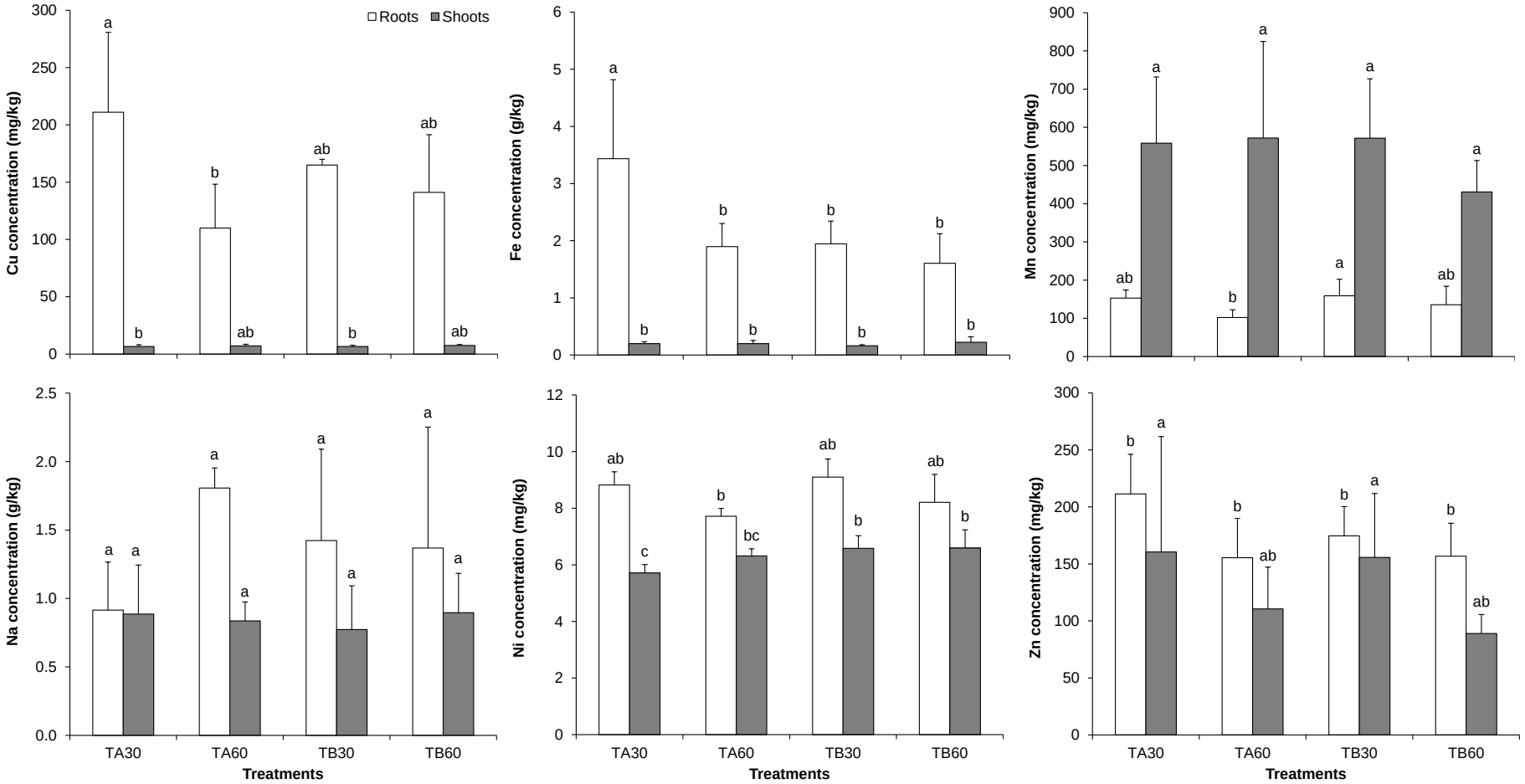

Fig. 6 Effects of the Technosols on the concentration of micronutrients in the roots and shoots of *Lavandula pedunculata*. Values from same organ and element followed by a different letter are significantly different ($p < 0.05$).

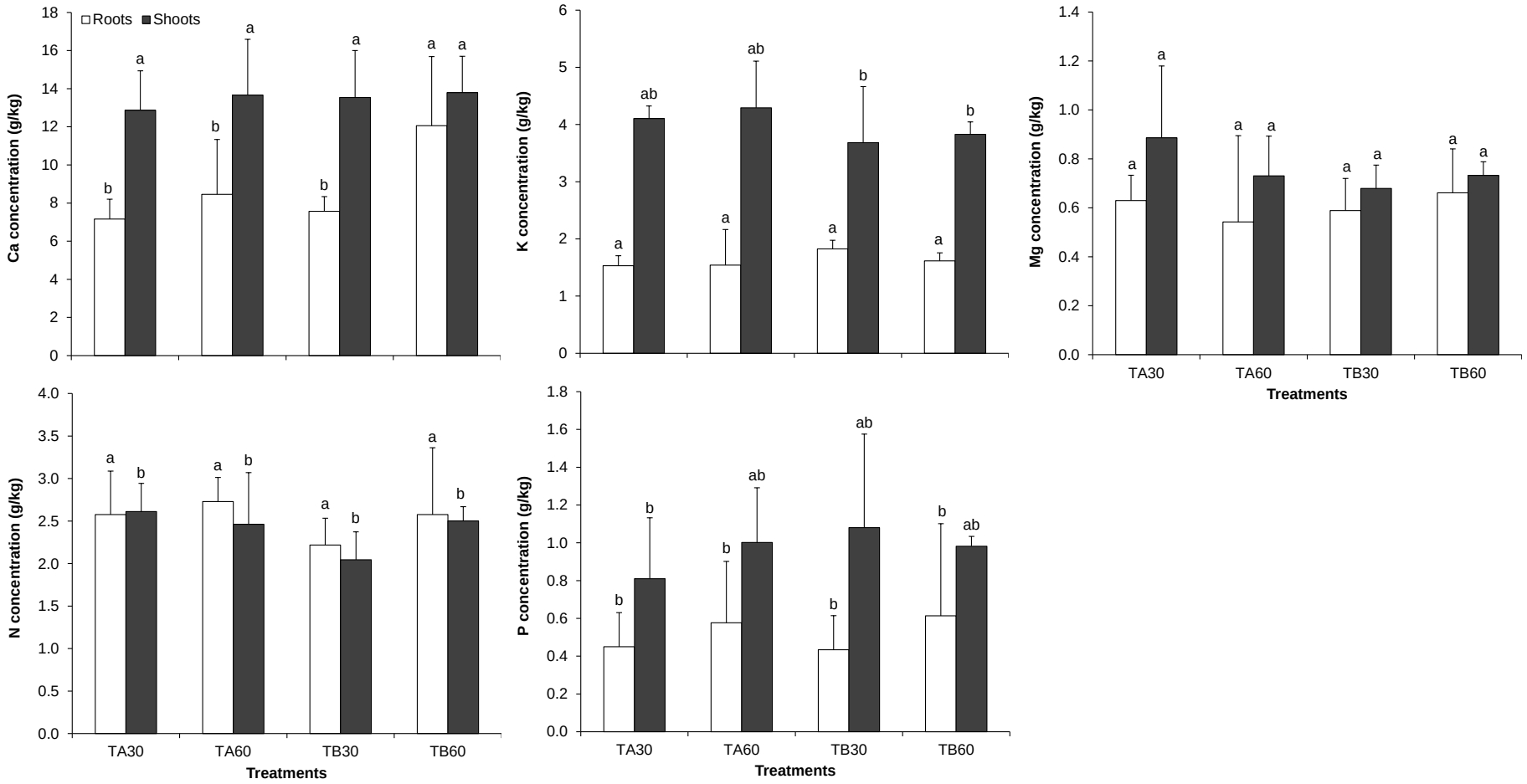

Fig. 7 Effects of the Technosols on the concentration of macronutrients in the roots and shoots of *Cistus ladanifer*. Values from same organ and element followed by a different letter are significantly different ($p < 0.05$).

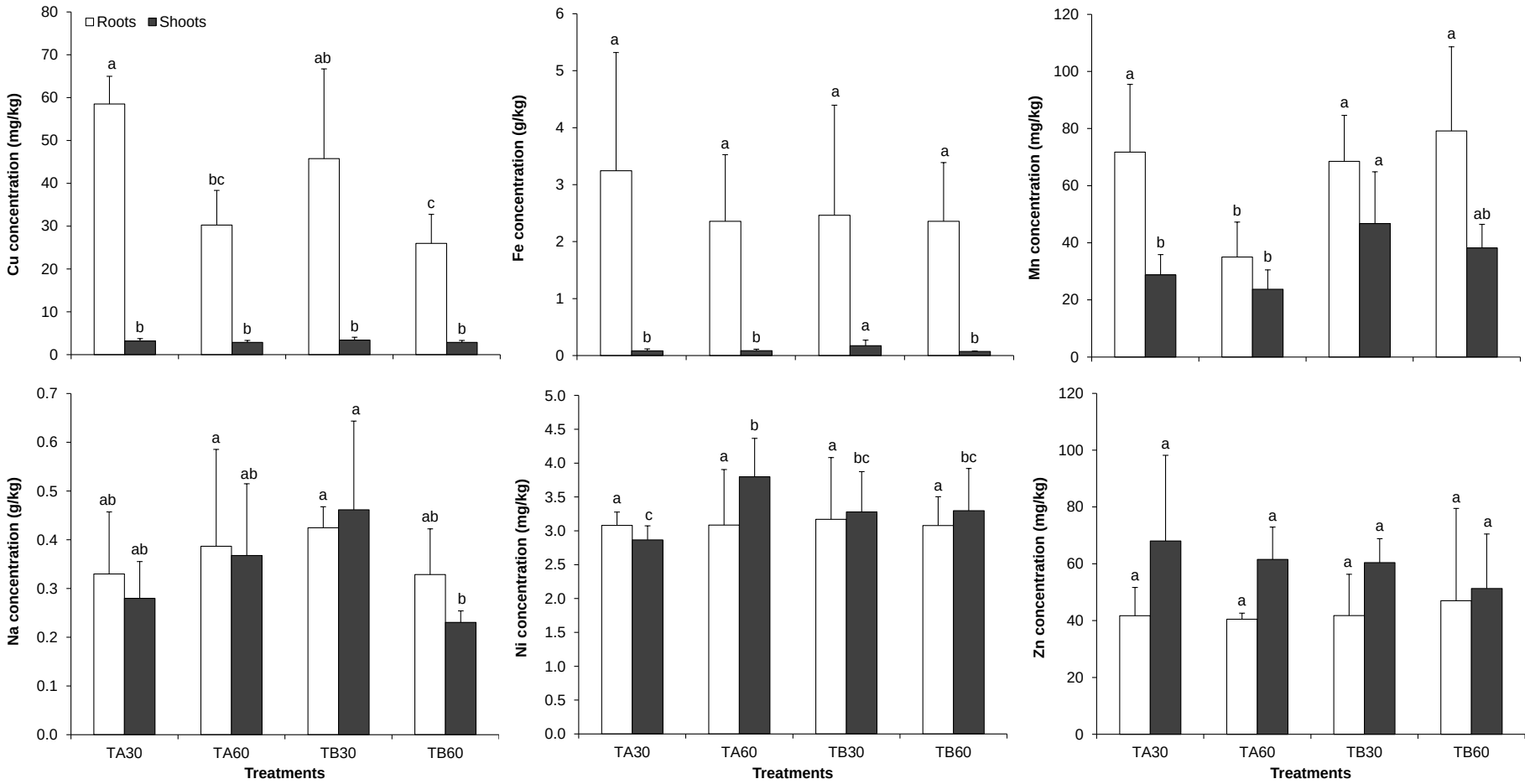

Fig. 8 Effects of the Technosols on the concentration of micronutrients in the roots and shoots of *Cistus ladanifer*. Values from same organ and element followed by a different letter are significantly different ($p < 0.05$).

Both species were accumulators of macronutrients (TransferC > 1) and non-accumulators of micronutrients. Exception was obtained for Na in *L. pedunculata,* which presented accumulation behaviour in all the Technosols (TransferC: 0.6−1.8), while *C. ladanifer* was non-accumulator of Na (TransferC < 0.9).

Variability intra and inter-population of ecological behaviours and/or elements concentrations in the two studied species growing spontaneously in mining areas are reported by several authors (de la Fuente et al., 2010; Freitas et al., 2004b; Santos et al., 2012, 2014a and references therein). Differences in the concentrations of some nutrients obtained in this study can related to seasonal and temporal variations in plants and the analysed plant part. The lower nutrients concentrations in plants growing in the Technosols, compared to plants from the field, can be attributed to the high availability of nutrients in the substrata.

Efficiency of the combined rehabilitation system

The Technosols studied ensure the germination and good vegetative development of the species, *C. ladanifer*and *L. pedunculata*. Biomass ashes from the barrier located in the bottom of the Technosols can supply some macro- and micronutrients (Table 2) to the plants and minimize their drought stress due to its significant water holding capacity (Ram and Masto, 2010). The plants did not show visual signs of phytotoxicity and/or deficiency of any elements, independently of the Technosols where grown.

The species studied had sustainable characteristics/behaviours (high uptake of potentially hazardous elements, indicated by BAC; storage of hazardous elements in the roots, indicated by TranslC; non-accumulator behaviour of potentially hazardous elements, indicated by TransfC; good ground cover; perennial; deeply root system), to their use in phytostabilisation programs of mine wastes. All these characteristics are reported by Mendez and Maier (2008) as the main requirements for a sustainable phytoremediation program. The referred behaviours of the studied plants were similar to those of the same species growing in natural conditions. Additionally, elements concentrations obtained in the shoots from plants growing in the Technosols (Figs. 4−8) were under the metal toxicity limits for domestic animals (NCR, 2005) that, occasionally, stay in São Domingos mining area (cattle and sheep). So, the implementation of a phytostabilisation program with the studied Technosols and the cultivation of both plant species do not pose any environmental risk.

Phytostabilisation programs have low costs of installation and maintenance. Although the available fractions of the majority of the nutrients were not different among Technosols, the application of the high amendments dose to the GW highly improved some of its chemical characteristics, as is the case of: concentration of organic C; extractable P; Mn in *L. pedunculata* assay and Ca in *C. ladanifer* assay (Figs. 2 and 3, and Table 3). Thus, the conception of a Technosol with the high amendments dose can reflect a significant difference in the economic cost of the phytostabilisation process, even only considering the transportation of organic/inorganic wastes. Nonetheless the present study, as well as others done with same species and similar mine wastes (Santos et al., 2014b, 2015), showed that the Technosols containing more amounts of amendments mixture can guarantee the sustainability of the

rehabilitation process during longer periods. Additionally, the improvement of the fertility was sufficient to increase the growth of the two studied species which have a significant economic value to cosmetic and pharmaceutical (Barrajón-Catalán et al., 2010; Cavanagh and Wilkinson, 2002) as well as other industrial purposes like cleaning products or pellets. Therefore, the increase of the shoots biomass production can contribute to a greater economic income and, consequently, the recovery of the rehabilitation cost. It is essential the assessment of the public safety risk of these products derived from contaminated areas.

The usual rehabilitation strategy of tailings, especially those composed of reactive minerals like sulfides, involves the covering. According to Santos et al. (2014c, 2015), the maintenance of moist SW from São Domingos mine generates very acidic aqueous leachates containing several elements, which ascend to the surface by capillarity during the hot periods and then originates several crystals, which can be more or less readily dissolved after wetting. The barrier between the sulfide wastes (SW) and the Technosols, composed of limestone and biomass ashes, which was tested in this study, neutralized the acidic solutions and provided an efficient alternative in the SW coverage and, consequently, reduction of its oxidation. The absence of salt efflorescences on the surface of the materials (GW or Technosols) and maintenance of pH values in biomass ashes and surface materials (Table 6) confirm the absence of this process.

In general, precipitation of secondary solid phases on the surface of limestone can form a coating that limit or avoid the interaction with the adjacent aqueous solution (Pérez-López et al., 2007; Santos et al., 2014c). However, in the present study the combined use of limestone and the biomass ashes seems to extend the efficiency of the alkalinity potential of the barrier, especially of the limestone. This barrier still contributed to buffer SW materials even for three years due to, possibly, eluviation of small particles of biomass ashes and lixiviation of carbonates, especially, from limestone and biomass ashes. In fact, pH values of SW located below the barrier were more than twice (Control: 3.8; TA30: 4.7; TA60: 3.7; TB30: 4.6; TB60: 3.5) the initial value of SW (Table 2). Laboratory experiments conducted by Pérez-López et al. (2009) show that the microencapsulation of pyrite-rich sludge by formation of Fe-coatings, due to fly ashes effect, inhibited sulfide oxidation.

Both materials from the barrier also lead to the retention of Al, As, Cu, Fe, Na, Pb, S and Zn resulting in the significant increase of their concentrations (Table 6) compared to the initial values (Table 2). Precipitation as metal-oxyhydroxides and/or carbonates, as a result of the increase of pH, can be the main mechanism of immobilisation of those elements when acidic aqueous solutions ascend from SW. In fact, several authors stated that amending contaminated wastes with liming materials and fly ashes reduce the availability of several elements (Bolan et al., 2014; Pérez-López et al., 2007; Ram and Masto, 2010; Su and Wong, 2003). Additional advantage of biomass ashes in the system is the formation of Fe-oxyhydroxides as a result of ash-aqueous solutions interactions (Ram and Masto, 2010), which are important components for absorption and fixation of some elements, namely As (Bolan et al., 2014; Kabata-Pendias, 2011; Kumpiene et al., 2008). At end of one and three years, it was not observed any hardpan formation in the contact zone between the barrier and SW as described by Pérez-López et al. (2007) in a column assay where a top layer of fly ashes covers pyritic sludge.

Table 6 Total concentration of the elements and pH in the materials from different layers collected at the end of the assay (three years) with *Cistus ladanifer*.

	pH	g/kg														
		Al	**As**	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Ni**	**P**	**Pb**	**S**	**V**	**Zn**
							Gossan wastes (Control)									
$GW_{(0\text{-}5cm)}$	4.1	9.5	8.80	1.0	0.18	208	2.1	0.2	0.04	0.6	8×10^{-3}	0.30	28.80	7.0	0.05	0.10
$GW_{(5\text{-}10cm)}$	4.2	11.2	9.01	0.3	0.18	232	2.2	0.2	0.03	0.6	5×10^{-3}	0.34	29.20	6.9	0.05	0.08
$GW_{(15\text{-}20cm)}$	7.3	10.4	8.90	1.3	0.18	226	2.0	0.2	0.05	0.4	9×10^{-3}	0.33	26.90	6.4	0.06	0.09
BA	8.4	29.6	1.30	120	0.18	48.3	11.4	9.5	1.87	3.0	0.07	2.75	3.89	7.0	0.07	0.29
L	—	5.7	0.08	331	0.20	6.6	1.6	2.9	0.08	0.2	0.01	0.04	4.37	11.1	9×10^{-3}	0.18
							TA60									
$TA60_{(0\text{-}5cm)}$	5.9	8.2	8.38	3.1	0.16	208	1.7	0.7	0.08	0.8	9×10^{-3}	0.59	25.60	5.9	0.06	0.10
$TA60_{(5\text{-}10cm)}$	5.8	11	8.85	2.8	0.17	224	2.0	0.7	0.08	0.7	9×10^{-3}	0.70	27.00	6.3	0.06	0.10
$TA60_{(15\text{-}20cm)}$	7.8	12.3	9.50	6.4	0.18	204	2.3	0.9	0.12	0.7	0.01	0.73	30.90	7.5	0.06	0.15
BA	8.1	33.6	0.39	131	0.39	29	12.7	9.8	2.17	3.5	0.09	2.75	1.07	4.4	0.07	0.46
L	—	8.0	0.08	308	0.20	8.9	2.1	2.8	0.07	0.2	7×10^{-3}	0.07	3.17	13.0	0.01	0.13
							TB30									
$TB30_{(0\text{-}5cm)}$	5.7	7.9	8.34	2.4	0.15	213	1.5	0.4	0.06	0.4	6×10^{-3}	0.77	25.60	5.6	0.05	0.08
$TB30_{(5\text{-}10cm)}$	5.7	11.1	8.95	1.9	0.17	234	1.9	0.6	0.06	1.0	9×10^{-3}	0.51	26.60	6.0	0.06	0.09
$TB30_{(15\text{-}20cm)}$	8.1	11.4	8.28	5.7	0.17	210	2.2	0.7	0.11	0.9	0.01	0.59	25.50	5.9	0.06	0.09
BA	8.5	32.9	0.53	127	0.21	30.4	10.6	10.3	2.06	3.0	0.08	2.56	1.54	1.9	0.07	0.27
L	—	5.8	0.05	323	0.12	4.7	1.3	3.0	0.08	0.2	8×10^{-3}	0.04	4.14	5.4	8×10^{-3}	0.08

BA: Biomass ashes; L: Limestone; TA60: Technosol containing GW and AgW+AW+RW at 60 g/kg; TB30: Technosol containing GW and AgW+CW+RW at 30 g/kg; GW: *gossan* wastes; AgW: agriculture wastes; AW: residue from the liquor distillation of *A. unedo* fruit; CW: residue from liquor distillation of *C. siliqua* fruit; RW: rockwool used for strawberry crops

The tested composed barrier has the advantage of being porous, allowing the rainwater leaching and reducing the flow along the barrier. Biomass ashes had also a significant moisture retention and water-holding capacity (Ram and Masto, 2010) reducing the leaching to deeper layers and, consequently, sulfides oxidation. This fact can still be improved by the plant development in the Technosols covering the barrier and forming the top layer of the entire system, which increases the evapotranspiration decreasing the water infiltration (Tordoff et al., 2000). Indeed, SW layer in control treatment (GW without plant due to plant death) was moister than those in treatments with Technosols and plant growth (data not shown).

CONCLUSION

The system of combined rehabilitation of *gossan* and sulfide-rich wastes was efficient and sustainable for long-term. The study demonstrated that the components of the barrier (limestone + biomass ashes) stabilise the sulfide wastes decreasing/preventing their oxidation and the ascension of the acid solutions rich in elements. Moreover this system allowed the rehabilitation of the sulfide rich wastes, which are not naturally colonized by vegetation, decreasing their erosion and generation of acid drainage. However, the top layer of *gossan* wastes without amendments addition (control) was not adequate to support the plants growth. The conception of Technosols is crucial to the implementation of the phytostabilisation programs in *gossan* wastes. In fact, Technosols made with *gossan* and agro-industrial wastes improved the chemical characteristics of the materials allowing the germination and establishment of *Cistus ladanifer* and *Lavandula pedunculata*. These plant species presented good development and ecological behaviours (uptake, translocation and accumulation) adequate to their use in this rehabilitation system. Co-cropping of the two plant species with contrasting nutritional abilities could improve phytostabilisation efficiency. The high biomass yields of both species can contribute with economic income due to their potential use in several industries. Assessment and monitoring should be made in order to ensure that toxic substances are not transferred to the plant-based products. Next step should be the implementation of the system in the field in order to confirm their efficacy under Mediterranean climate conditions.

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REFERENCES

Abreu, M.M., Magalhães, M.C.F., 2009. Phytostabilization of soils in mining areas. Case studies from Portugal. In: Aachen, L., Eichmann, P. (Eds.), Soil Remediation. Nova Science Publishers, Inc., New York, pp. 297-344.

Abreu, M.M., Tavares, M.T., Batista, M.J., 2008. Potential use of *Erica andevalensis* and *Erica australis* in phytoremediation of sulphide mine environments: São Domingos, Portugal. Journal of Geochemical Exploration 96(2-3), 210-222.

Abreu, M.M., Santos, E.S., Magalhães, M.C.F., Nabais, C., 2009. Arsenic containing phases in soils from São Domingos mining area and in non-contaminated soils from Pomarão and Serra do Caldeirão. Revista de Ciências Agrárias 31(1), 155-169.

Abreu, M.M., Batista, M.J., Magalhães, M.C.F., Matos, J.X., 2010. Acid mine drainage in the Portuguese Iberian Pyrite Belt. In: Robinson, Brock C. (Ed.), Mine drainage and related problems. Nova Science Publishers, Inc., New York, pp. 71-118.

Abreu, M.M., Santos, E.S., Ferreira, M., Magalhães, M.C.F., 2012a. *Cistus salviifolius* a promising species for mine wastes remediation. Journal of Geochemical Exploration 113, 86-93.

Abreu, M.M., Santos, E.S., Fernandes, E., Magalhães, M.C.F., 2012b. Trace elements tolerance, accumulation and translocation in *Cistus populifolius*, *Cistus salviifolius* and their hybrid growing in polymetallic contaminated mine areas. Journal of Geochemical Exploration 123, 52-60.

Activation Laboratories, 2014. 1H – Total Digestion – ICP, INAA. Available in: www.actlabs.com/page.aspx?page=506&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes2010 (Accessed January 2014)

Adriano, D.C., 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability and risk of metals. New York, Springer-Verlag.

Adriano, D.C., Wenzel, W.W., Vangronsveld, J., Bolan, N.S., 2004. Role of assisted natural attenuationin environmental cleanup. Geoderma 122, 121-142.

Alvarenga, P., Palma, P., Gonçalves, A.P., Baião, N., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008a. Assessment of chemical, biochemical and ecotoxicological aspects in a mine soil amended with sludge of either urban or industrial origin. Chemosphere 72, 1774-1781.

Alvarenga, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008b. Evaluation of composts and liming materials in the phytostabilization of a mine soil using perennial ryegrass. Science of the Total Environment 406, 43-56.

Álvarez-Valero, A.M., Pérez-López, R., Matos, J., Capitán, M.A., Nieto, J.M., Sáez, R., Delgado, J., Caraballo, M., 2008. Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian Peninsula): evidence from a chemical and mineralogical characterization. Environmental Geology 55(8), 1797-1809.

Arbestain, M.C., Madinabeitia, Z., Hortala, M.A., Macías-García, F., Virgel, S., Macías, F., 2008. Extractability and leachability of heavy metals in Technosols prepared from mixtures of unconsolidated wastes. Waste Management 28, 2653-2666.

Barrajón-Catalán, E., Fernández-Arroyo, S., Roldán, C., Guillén, E., Saura, D., Segura-Carretero, A., Micol, V. 2011. A Systematic study of the polyphenolic composition of aqueous extracts deriving from several *Cistus* genus species: evolutionary relationship. Phytochemical Analysis 22, 303-312.

Bolan, N., Kunhikrishnan, A., Thangarajan, R., Kumpiene, J., Park, J., Makino, T., Kirkham, M.B., Scheckel, K., 2014. Remediation of heavy metal(loid)s contaminated soils – To mobilize or to immobilize?. Journal of Hazardous Materials 266,141-166.

Bolaños, D.R., Romero, N., Macías-García, F., Nieto, C., Pérez, C., Verde, J.R., Macías, F., 2015. Recuperación de la mina de Touro con aplicación de Tecnosoles y humedales reactivos. Proceedings XX Congreso Latinoamericano y XVI Congreso Peruano de la Ciencia del Suelo, 8 pp.

Burgos, P., Madejón, P., Cabrera, F., Madejón, E., 2010. By-products as amendment to improve biochemical properties of trace element contaminated soils: effects in time. International Biodeterioration and Biodegradation 64, 481-488.

Cavanagh, H.M.A., Wilkinson, J.M., 2002. Biological activities of Lavender essential oil. Phytotherapy Research 16, 301-308.

CCME − Canada Council of Ministers of the Environment, 2007. Canadian soil quality guidelines for the protection of environmental and human health: summary tables (Updated September, 2007). CCME, Winnipeg, Canada.

Clemente, R., Concepción, A., Bernal, M.P., 2006. A remediation strategy based on active phytoremediation followed by natural attenuation in a soil contaminated by pyrite waste. Environmental Pollution 143(3), 397-406.

Corral, R., Pita, J.M., Pérez-García, F., 1990. Some aspects of seed germination in four species of *Cistus* L. Seed Science and Technology 18, 321-325.

de la Fuente, V., Rufo, L., Rodríguez, N., Amils, R., Zuluaga, J., 2010.Metal accumulation screening of the Río Tinto Flora (Huelva, Spain). Biological Trace Element Research 134, 318-341.

De Varennes, A., Abreu, M.M., Qum G., Cunha-Queda, C., 2010. enzymatic activity of a mine soil varies according to vegetation cover and level of compost applied. International Journal of Phytoremediation 12(4), 371-383.

EPA − United States Environmental Protection Agency, 2000. Introduction to phytoremediation. EPA/600/R-99/107.

Feng, M., Shan, X., Zhang, S., Wen, B., 2005. A comparison of rhizosphere-based method with DTPA, EDTA, $CaCl_2$ and $NaNO_3$ extraction methods for prediction of bioavailability of metals in soil to barley. Environmental Pollution 137, 231-240.

Freitas, H., Prasad, M.N.V., Pratas, J., 2004a. Plant community tolerant to trace elements growing on degraded soils of São Domingos mine in the south east of Portugal: environmental implications. Environment International 30, 65-72.

Freitas, H., Prasad, M.N.V., Pratas, J., 2004b. Analysis of serpentinophytes from north–east of Portugal for trace metal accumulation − relevance to the management of mine environment. Chemosphere 54, 1625-1642.

INIA–LQARS, 2000. Manual de Fertilização das culturas. Laboratório Químico Agrícola Rebelo da Silva (Ed.), Lisboa.

Kabata-Pendias, A., 2011. Trace elements in soils and plants, 4th edition. CRC Press. Taylor & Francis Group, Boca Raton.

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments – A review. Waste Management 28, 215-225.

Macías, F., 2004. Recuperación de suelos degradados, reutilización de resíduos y secuestrode carbono. Una alternativa integral de mejora de la calidad ambiental. Recursos Rurais 1, 49-56.

Macías, F., Macías-García, F., Nieto, C., Verde, J.R., Pérez, C., Bao, M., Camps-Arbestain, M., 2011. Gestión de residuos y cambio climático. In: Mosquera, M.E.L., Osés, M.J.S. (Eds.), Gestión de residuos orgánicos de uso agrícola. Servizo de Publicacións e Intercambio Científico de la Universidade de Santiago de Compostela, Santiago de Compostela.

Macías-García, F., Fontán, L., Otero, X.L., Llaguno, C.P., Arbestain, M.C, Macías, F., 2009. Recuperación de aguas ácidas de la mina Touro mediante sistemas integrados de barreras reactivas con diferentes Tecnosoles y humedales, in: Minería Sostenible. Cámara Oficial Mineira de Galicia, A Coruña. pp. 963-973.

Matos, J.X., Martins, L.P., 2006. Reabilitação ambiental de áreas mineiras do sector português da Faixa Piritosa Ibérica: estado da arte e perspectivas futuras. Boletín Geológico y Minero 117, 289-304.

Matos, J.X., Pereira, Z., Oliveira, V., Oliveira, J.T., 2006. The geological setting of the São Domingos pyrite orebody, Iberian Pyrite Belt. Actas do VII Congresso Nacional de Geologia, 4 pp.

Mendez, M.O., Maier, R.M., 2008. Phytoremediation of mine tailings in temperate and arid environments. Reviews in Environmental Science and Biotechnology 7, 47-59.

Monterroso, C., Macías, F., Gil Bueno, A., Val Caballero, C., 1998. Evaluation of the land reclamation project at the As Pontes Mine (NW Spain) in relation to the suitability of the soil for plant growth. Land Degradation & Development 9, 441-451.

NCR − National Research Council, 2005. Mineral tolerance of animals. National Academies Press, Washington.

Perelman, A.I., 1966. The geochemistry of land areas in Russian. IzdVishShk, Moscow. In: Nagaraju, A., Karimulla, S., 2002. Accumulation of elements in plants and soils in and around Nellore Mica Belt, Andhra Pradesh, India – a biogeochemical study. Environmental Geology 41, 852-860.

Pérez-de-Mora, A., Madejón, P., Burgos, P., Cabrera, F., Lepp, N.W., Madejón, E., 2011. Phytostabilization of semiarid soils residually contaminated with trace elements using by-products: Sustainability and risks. Environmental Pollution 159, 3018-3027.

Pérez-López, R., Nieto, J.M., Almodóvar, G.R., 2007. Immobilization of toxic elements in mine residues derivedfrom mining activities in the Iberian Pyrite Belt (SW Spain): Laboratory experiments. Applied Geochemistry 22, 1919-1935.

Pérez-López, R., Álvarez-Valero, A.M., Nieto, J.M., Sáez, R., Matos, J.X., 2008. Use of sequential extraction procedure for assessing the environmental impact at regional scale of the São Domingos Mine (Iberian Pyrite Belt). Applied Geochemistry 23, 3452-3463.

Pérez-López, R., Cama, J., Nieto, J.M., Ayora, C., Saaltink, M.W., 2009. Attenuation of pyrite oxidation with a fly ash pre-barrier: Reactive transport modelling of column experiments. Applied Geochemistry 24, 1712-1723.

Pérez-López, R., Márquez-García, B., Abreu, M.M., Nieto, J.M., Córdoba, F., 2014. *Erica andevalensis* and *Erica australis* growing in the same extreme environments: Phytostabilization potential of mining areas. Geoderma 230-231, 194-203.

Qu, G., de Varennes, A., 2010. Use of hydrophilic polymers from diapers to aid the establishment of *Spergularia purpurea* in a mine soil. Journal of Hazardous Materials 178, 956-962.

Quental, L., Bourguignon, A., Sousa, A.J., Batista, M.J., Brito, M.G., Tavares, T., Abreu, M.M., Vairinho, M., Cottard, F., 2002. MINEO Southern Europe environment test site. Contamination impact mapping and modelling - Final Report. Assessing and monitoring the environmental impact of mining activities in Europe using advanced Earth Observation Techniques (MINEO) 5yth FP-IST-1999-10337.

Ram, L,C., Masto, R.E., 2010. An appraisal of the potential use of fly ash for reclaiming coal mine spoil. Journal of Environmental Management 91, 603-617.

Santibáñez, C., Verdugo, C., Ginocchio, R., 2008. Phytostabilization of copper mine tailings with biosolids: Implications for metal uptake and productivity of *Lolium perenne*. Science of the Total Environment 395, 1-10.

Santos, E.S., Abreu, M.M., Nabais, C., Saraiva, J., 2009.Trace elements and activity of antioxidative enzymes in *Cistus ladanifer* L. growing on an abandoned mine area. Ecotoxicology 18, 860-868.

Santos, E.S., Abreu, M.M., Nabais, C., Magalhães, M.C.F., 2012. Trace elements distribution in soils developed on *gossan* mine wastes and *Cistus ladanifer* L. tolerance and bioaccumulation. Journal of Geochemical Exploration 123, 45-51.

Santos, E.S., Abreu, M.M., de Varennes, A., Macías, F., Leitão, S., Cerejeira, M.J., 2013a. Evaluation of chemical parameters and ecotoxicity of a soil developed on *gossan* following application of polyacrylates and growth of *Spergularia purpurea*. Science of the Total Environment 461-462, 360-370.

Santos, E.S., Nabais, C., Abreu, M.M., 2013b. Adaptabilidade ecofisiológica de diferentes populações de *Cistus ladanifer* L. do sul de Portugal. STUDIA – Scientiæ Rerum Diffusio: Suplemento Temático IV Seminário Luso-brasileiro em Ciências do Ambiente e Empresariais, 6 pp

Santos, E.S., Abreu, M.M., Batista, M.J., Magalhães, M.C.F., Fernandes, E., 2014a. Inter-population variation on the accumulation and translocation of potentially harmful chemical elements in *Cistus ladanifer* L. from Brancanes, Caveira, Chança, Lousal, Neves Corvo and São Domingos mines in the Portuguese Iberian Pyrite Belt. Journal of Soils and Sediments 14, 758-772.

Santos, E.S., Abreu, M.M., Macías, F., de Varennes, A., 2014b. Improvement of chemical and biological properties of *gossan* mine wastes following application of amendments and growth of *Cistus ladanifer* L.. Journal of Geochemical Exploration 147, 173-181.

Santos, E.S., Magalhães, M.C.F., Abreu, M.M., Macías, F., 2014c. Effects of organic/inorganic amendments on trace elements dispersion by leachates from sulfide-containing tailings of the São Domingos mine, Portugal. Time evaluation. Geoderma 226-227, 188-203.

Santos, E.S., Abreu, M.M., Macías, F., de Varennes, A., 2015. Chemical quality of leachates and enzimatic activities in Technosols with *gossan* and sulfide wastes from the São Domingos mine. Journal of Soils and Sediments DOI: 10.1007/s11368-015-1068-8

Su, D.C., Wong, J.W.C. 2003. Chemical speciation and phytoavailability of Zn, Cu, Ni and Cd in soil amended with fly ash-stabilized sewage sludge. Environment International 29, 895-900.

Tordoff, G.M., Baker, A.J.M., Willis, A.J., 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere 41, 219-228.

Tu, S., Ma, L., Luongo, T., 2004. Root exudates and arsenic accumulation in arsenic hyperaccumulating *Pteris vittata* and non-hyperaccumulating *Nephrolepis exaltata*. Plant and Soil 258, 9-19.

USEPA − United States Environmental Protection Agency, 1979. Methods for chemical analysis of water and waste. Office of Research and Development, US Environmental Protection Agency, Cincinatti, Ohio (method 365.2).

Wong, M.H., 2003. Ecological restoration of mine degraded soils with emphasis on metal contaminated soils. Chemosphere 50, 775-780.

Yao, F.X., Macías, F., Santesteban, A., Virgel, S., Blanco, F., Jiang, X., Camps-Arbestain, M., 2009. Influence of the acid buffering capacity of different types of Technosols on the chemistry of their leachates. Chemosphere 74, 250-258.

Zhang, S., Li, W., Shan, X.Q., Lu, A.,Z hou, P., 2005. Effects of low molecular weight organic anions on the release of arsenite and arsenate from a contaminated soil. Water, Air and Soil Pollution 167(1-4), 111-122.

Zanuzzi, A., Arocena, J.M., van Mourik, J.M., Faz Cano, A., 2009. Amendments with organic and industrial wastes stimulate soil formation in mine tailings as revealed by micromorphology. Geoderma 154, 69-75

11. EVALUATION OF CHEMICAL PARAMETERS AND ECOTOXICITY OF A SOIL DEVELOPED ON GOSSAN FOLLOWING APPLICATION OF POLYACRYLATES AND GROWTH OF SPERGULARIA PURPUREA

ABSTRACT

The aim of this study was to evaluate the chemical characteristics and ecotoxicity of a mine soil developed on *gossan* materials and amended with hydrophilic polyacrylate polymers after a growth cycle of *Spergularia purpurea*. Different acute bioassays (*Daphnia magna* immobilization; microalgae growth inhibition; germination and growth of lettuce and oat) were carried out with simulated leachates, pore water and soil samples. The germination and growth of native shrubs (*Cistus ladanifer* and *Lavandula sampaioana* were also evaluated in the lysimeters where *S. purpurea* had grown.

The soil had high total concentrations (g/kg) of Al (3.50–8.60), As (2.55–2.73), Cu (0.13–0.91) and Pb (4.48–6.16). However, the percentages of elements in aqueous extracts (simulating leachates, pore water, and the conditions of the rhizosphere soil) were small when compared to their total soil concentrations (less than 9 % except for Na in leachates). Growth of *S. purpurea* and other natural colonization of plant species (Poaceae, Fabaceae and Asteraceae families) improved chemical characteristics but the application of the polyacrylate polymers contributed to a further improvement of soil quality. However, this was not sufficient to ensure the growth of a large number of shrubs despite a great germination rate.

Among the several species used on the ecotoxicological assessment, the *D. magna* test was the only bioassay that showed a clear toxicity of soil leachates, suggesting the importance of using several ecotoxicological tests to assess the environmental risk of soil contamination and its rehabilitation.

Although the studied soil can be considered contaminated taking into account the total soil concentrations of Al, As, Cu and Pb, the low concentrations of the same chemical elements in extractable solutions, that simulated the fractions really available for organisms, did not demonstrate a substantial toxic effects in the organisms and, consequently, negative impact on the environment.

KEYWORDS São Domingos mine • Bioassays • Leachates • Pore water • Rizosphere solution

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INTRODUCTION

Abandoned mining areas are a significant problem in the Iberian Pyrite Belt (IPB). São Domingos is an abandoned copper mine located in the Portuguese part of the IPB (SE of Portugal) that is said to present a high environmental risk (Matos and Martins, 2006). Weathering of the sulfides-rich mineralization was responsible for the generation of a significant *gossan*, which was intensely exploited in Roman times. Modern exploitation (middle of the XIX century until 1960) occurred in both *gossan* and massive sulfides containing As, Cu, Pb and Zn (Quental et al., 2002). The mining wastes generated, over the long period of extraction and transformation of the ore, represent an important source of acidity and of several trace elements with the consequent contamination of adjacent areas, including superficial waters and soils/sediments/host rocks (Abreu et al., 2010; Álvarez-Valero et al., 2008).

A large amount of organic and inorganic wastes are produced daily and disposed of in landfills, although several wastes can be used for the rehabilitation of degraded and contaminated areas, due to their chemical composition, provided they are available in sufficient amounts and close to the target area (Macías, 2004).

Large quantities of hygiene products (diapers, paper towels, feminine products) containing polyacrylate polymers are used and they occupy a large volume in landfills. However, this type of wastes was shown to increase the growth of grasses and enhance some soil enzymatic activities (Al-Humaid and Moftah, 2007; Boatright et al., 1997; de Varennes et al., 1999; Guiwei et al., 2008; Qu and de Varennes, 2010). Moreover, due to the increase of the water-holding capacity of the soil, the use of these polymers can be especially useful in soils in the Mediterranean region as the risk of drought is high and periods without rain are frequent (Silberbush et al., 1993).

Total soil concentrations of chemical elements are used as guidelines to establish whether a soil should be considered as contaminated, although only the elements in soil solution and/or exchangeable positions associated with inorganic and organic colloids are bioavailable (Adriano, 2001; Kabata-Pendias, 2011). Thus, in remediation processes it is important to determine the concentrations of chemical elements in soil leachates and available fractions (extracted with several aqueous solutions: buffered and unbuffered salt solutions, acid solutions, chelating agents, etc.; Kabata-Pendias, 2004). The evaluation of ecotoxicological effects of the application of amendments should also be carried out, as chemical analysis per se is insufficient to evaluate potential ecological risks (van Gestel et al., 2001).

Any strategy to assess the potential risk of a soil should include tests with organisms of different trophic levels, from the terrestrial and aquatic compartments (Bagur-González et al., 2011; García-Lorenzo et al., 2009; Knoke et al., 1999). Bioassays with vascular plants offer a variety of advantages to evaluate soil toxicity, allowing the assessment of a large array of parameters that can be affected by hazardous elements (Ferrari et al., 1999; van Gestel et al., 2001). Bioassays using aquatic organisms have been used to evaluate the ecotoxicity in several ecosystems (Cerejeira et al., 1998; Pereira et al., 2000; Silva et al., 2012) even in mining areas from IPB (Alvarenga et al., 2012, 2008; Rocha et al., 2011).

The aim of this study was to evaluate the chemical characteristics and ecotoxicity of a soil developed on *gossan* materials after amendment with hydrophilic polyacrylate polymers and growth of *Spergularia purpurea* (Pearson) G. Don fil..The natural colonization by herbaceous species and, germination and development of native shrub species (*Cistus ladanifer* L. and *Lavandula sampaiona* (Rozeira) Rivas Mart., T.E. Díaz & Fern.Gonz.) were also evaluated to better understand the post-rehabilitation period.

MATERIALS AND METHODS

Sampling area

The sampling area was located within the São Domingos mining area, to the north of the open pit (37º40'N, 70º30'W), where soils were developed on *gossan* materials and host rocks (Spolic Technosol Toxic; IUSS Working Group WRB, 2007). The vegetation present on the site included *Cistus ladanifer*, *Cistus salviifolius* L., *Lavandula luisieri* (Rozeira) Rivas-Martınez, *Lavandula sampaioana*, *Erica australis* L. and many herbaceous species. About 1000 kg of the top soil (0–20 cm) was collected in 2009 to be used in a mesocosm experiment. The soil was sandy and about 52 % consisted of host rock fragments coarser than 2 mm.

Mesocosm experiment

The experiment was performed in lysimeters (50 cm long with a diameter of 60 cm) with unsieved mine soil in four treatments (three replicates each): (1) control without polymer; (2) soil amended with 25 diapers cut into 5 cm pieces and incorporated into the top 25 cm of the soil (about 0.3 % m/m of polyacrylate polymer); (3) soil amended with the polyacrylate polymer removed from 25 diapers; and (4) soil amended with a polyacrylate polymer synthesized by Marion Roussel Ltd (polymer 7015) applied at the same rate as before. The preparation of the mesocosm experiment and the establishment of an indigenous grass (*Spergularia purpurea*) from the São Domingos area are described in detail by Qu and de Varennes (2010). Briefly, 2 L of synthetic urine was added to each lysimeter and, after two weeks of incubation, 10 L of deionized water per week was added during one month to simulate rainfall. After growth of S. purpurea (from April until early June 2009), the soil was left bare during the summer and then used for the present experiment.

Composite soils samples from the 0–7 cm layer were collected from each lysimeter (nine subsamples per lysimeter). Seeds of *C. ladanifer* (after a pre-treatment at 100 ºC for 30 minutes; Corral et al., 1990), and L. sampaioana, collected in the São Domingos mine in 2009, were sown (0.5 g of each species per lysimeter) in the lysimeters (October of 2009) immediately after soil sampling. Germination and growth of both shrubs were monitored over a 10 months period. Spontaneous species were also identified and their total fresh biomass evaluated at the end of the experiment.

Soil characterization

Initial soil, collected in the mine, and soil samples from each treatment were air-dried, homogenized and sieved. The soil fraction with particles less than 2 mm was characterized for (Póvoas and Barral, 1992): pH and electric conductivity (EC) in a water suspension (1:2.5 *m*/*V*); extractable K and P (Egner-Riehm method); total N (Kjeldahl method) and organic C by wet combustion. Nitric and ammoniacal N (Mulvaney, 1996) and Fe from total iron oxides (Tamm reagent with UV radiation; de Endredy, 1963) and from non-crystalline iron oxides (Tamm reagent in the dark; Schwertmann, 1964) were also determined.

Total concentrations of Al, As, Ca, Cu, Fe, K, Mg, Mn, Na, S, Pb, Zn from initial soil (fraction < 2 mm) were determined using ICP-OES and INAA, after acid digestion (perchloric acid + nitric acid + hydrochloric acid + hydrofluoric acid) in an international certified laboratory (Actlabs, ISO/IEC 17025, Activation Laboratories, 2012). The same chemical elements were analysed in three aqueous extractable solutions that simulated: leachates, the soil solution in the pore water, and the solution from rizosphere soil (RHIZO solution).

The leachates and pore waters were obtained at room temperature in a rotatory shaker in a solid-to-water ratio of 1:10 (*m*/*V*) during 24 h and five days (aerated daily), respectively (DIN 38414-S4, 1984; Buján et al., 2010). Then, these extractable solutions were filtrated under vacuum (< 0.45 µm), and the pH and EC were measured. The RHIZO solution was extracted with an aqueous diluted solution containing a mixture of organic acids (acetic acid + lactic acid + citric acid + malic acid + formic acid) after 16 h of agitation (Rhizosphere-based method, Feng et al., 2005). The filtrates were stored at –18 ºC until analysis for Al, Ca, Cu, Fe, K, Mg, Mn, Pb and Zn by flame atomic absorption spectrometry; As by graphite furnace atomic absorption spectrometry; chlorides, nitrates and sulfates by ion chromatography; and phosphates by visible spectrophotometry by the molybdenum blue method (USEPA, 1979).

Bioassays

Ecotoxicological evaluation of the leachates (indirect bioassays) was performed using two plant species (dicot – *Lactuca sativa* L. var. crispa L. cv. Great Lakes 118, and monocot – *Avena sativa* L. cv. Santa Eulália), a microalgae (*Pseudokirchneriella subcapitata* (Korshikov) Hindak) and a microcrustacean (*Daphnia magna* Straus). These bioassays evaluated the toxicity risk of the soil to adjacent areas by acting as a source of contamination (van Gestel et al., 2001). The same ecotoxicological tests were carried out on the soil with the same plant species to evaluate the soil matrix effect (direct bioassay).

Plants

Toxicity towards plants was assessed in accordance with the OECD Guideline 208 (OECD 208, 2006) by evaluating the effects on visual aspects, germination, root and shoot elongation and fresh

biomass of both plant species, following the exposure of the seeds and seedlings to moistened soil or leachates. Three bioassays were carried out: a filter paper test, a soil test and a hydroponic test. The seeds used were surface-sterilized in 10 % Na-hypochlorite solution for 20 min to prevent fungal growth and then washed with distilled water (Salvatore et al., 2008).

In one plant bioassay, three layers of filter paper (140 mm Watman No. 1 filter) were put on the bottom of glass beakers (100 mL) and moistened with 5 mL of leachate from each treatment (Filter paper test; Salvatore et al., 2008). Deionised water was used as the negative control. In a second bioassay, 15 g of the soil samples (fraction < 2 mm) were put in each glass beaker (Soil test; Martí et al., 2007).

Seven seeds of lettuce and five seeds of oat per replicate were placed in each beaker and then incubated in a growth chamber under controlled conditions (25 ± 1 ºC; 16 h light/ 8 h darkness). The filters and soils were kept moist and the germinated seeds were periodically counted during 15 days. The criterion of germination was the emergence of a radicle through the seed coat. Length of roots and shoots and fresh biomass were measured at the end of the experiment.

In a third bioassay (hydroponic test), lettuce and oat seeds were germinated in the dark at 25 ºC for 7 days and seedlings with a seminal root length of 50–80 mm and 15 mm respectively, (shoot length of 2–4 mm for lettuce and 30–50 mm for oat) were selected. Seedlings (seven of lettuce and five of oat in each replicate) were grown in beakers filled with 50 mL of leachates from the different treatments. The seedlings were supported by a thin and flexible plastic net placed on the top of each beaker, so that only the roots were immersed in the leachate. After 15 days of growth in the same conditions as before, the biological parameters described above were evaluated.

Microalgae and microcrustacean

Microalgae toxicity was assessed by measuring the growth inhibition of *P. subcapitata* exposed to leachates (100 % of concentration *V/V*) for 72 h, according to the AlgalToxKit FTM test procedure (MicroBioTests, 2004) that follows the OECD guideline 201 (OCDE, 1984). The microalgae was available as immobilized algal beads and 1 h prior to the test a concentrated algal inoculum of 1 x 106 algae/mL was prepared by de-immobilization of the algal beads by adding “Matrix dissolving Medium” and shaking vigorously every 2 min till complete dissolution. The test was performed using three replicates of all leachates on disposable 10 cm-long spectrophotometer cells (25 mL). At the end of the test, the total growth inhibition percentage was determined by converting optical densities (spectrophotometer with a 670 nm filter) into algal numbers using an exponential model. The microalgae test data analysis was performed through the determination of the average specific growth rate for each treatment followed by the calculation of the percentage inhibition (% I) for each test batch according to internationally accepted procedures (ISO 8692, 2004).

The microcrustacean acute toxicity was determined by the immobilisation of D. magna exposed to leachates for 48 h according to the bioassay procedure (MicroBio-Tests, 2000). The test organisms were hatched for 72 h at 20–22 ºC under continuous illumination of 6000 lux prior to the test performance from “dormant eggs (ephippia)”. At the beginning of the test, the young Daphnids

(neonates) were less than 24 h old. As for the microalgae test, all leachates from the mesocosm experiment were evaluated for their percentage of total effect using four replicates. At the end of the test, the immobilization percentages were calculated according to ISO 6341 (1996).

Data analysis

Data were analysed by a one way ANOVA and the Tukey test ($p < 0.05$) used to separate means, using the statistical programme SPSS v18.0 for Windows. For statistical purposes, the results below the detection limit were assumed as half of the detection limit. Bivariate Pearson correlations were used to correlate the soil and plant characteristics ($r > 0.80$). Quality control of the analysis was made by analytical replicate samples, use of certified standards solution and laboratory standards at the Activation Laboratories. All the analyses were performed in triplicate, except the total concentration of the chemical elements in initial soil which was done in duplicate.

The data from the bioassays using the leachates, the filter paper and both plant species were used to calculate two toxicity indexes reported by Bagur-González et al. (2011). The indexes can vary from −1 (maximum phytotoxicity) to > 0.

RESULTS AND DISCUSSION

Soil characterization

Initial soil from the São Domingos mine (before the mesocosm experiment) had chemical characteristics (Table 1) that are explained by the source materials and that can be considered as moderately extreme when compared to others *gossan* materials from this mining area (Abreu et al., 2012a,b; Santos et al., 2012) or contaminated soils from other mining areas in the IPB (Abreu et al., 2012a,b; Alvarenga et al., 2008; Rocha et al., 2011). Thus, initial soil was acid with low electrical conductivity (EC) and very low concentrations of organic C and extractable K and P. However, total N concentration was medium with a higher content of $N\text{-}NO_3$ than of $N\text{-}NH_4$. The enrichment of the São Domingos soil with iron oxides, in particular in the crystalline fraction, is related to the *gossan* materials.

The São Domingos soil can be considered contaminated with As, Cu and Pb (Table 2; CCME, 2007). Total soil concentrations of As, Cu, Pb, V and Zn are in the same range as those reported by Abreu et al. (2012a,b), Álvarez-Valero et al. (2008) and Santos et al. (2012) for *gossan* materials and soils developed on *gossan* from the São Domingos mining area.

No significant differences among all soil samples (treatments and initial soil) were observed for EC, extractable P and Fe in iron oxides, both crystalline and non crystalline fractions (Tables 1 and 3). Amended soils, independently of polyacrylate source, presented values of pH and organic C significantly higher than the control and the initial soil. The increase of soil pH following polymer application was observed previously and is related to the exchange of polymer counter ions (Na^+) with acidic ions in the soil (Qu and de Varennes, 2010). The pH values obtained by these authors were in the same range as those obtained after the four months of fallow, except for control which increased

from 4.7 to 5.9. The increase in organic C can be related to the presence of S. purpurea roots which were not collected in the previous study (Qu and de Varennes, 2010).

Table 1 Characteristics of the initial soil from the São Domingos mine (Mean ± SD; *n* = 3).

	Initial soil
pH (H_2O)	5.23 ± 0.25
Ec (µS/cm)	75.22 ± 33.20
Organic C (g/kg)	1.74 ± 0.16
N (mg/kg)	
Total	164.66 ± 43.23
N-NH_4	21.77 ± 9.92
N-NO_3	39.60 ± 14.31
Extractable P (mg/kg)	2.33 ± 0.60
Extractable K (mg/kg)	41.50 ± 14.75
Fe in iron oxides (g/kg)	
Non crystalline Fe oxides	0.13 ± 0.02
Crystalline Fe oxides	12.45 ± 3.41

Table 2 Total concentration of trace elements (g/kg Dry weight) in initial soils used to experiment from São Domingos mine (Mean ± SD; *n* = 2).

	Element concentration (g/kg Dry weight)	**Maximum allowed values[a]**
Al	6.05 ± 3.61	—
As	2.66 ± 0.13	0.01
Ca	0.25 ± 0.07	—
Cu	0.11 ± 0.03	0.06 – 0.09
Fe	67.30 ± 2.97	—
K	3.10 ± 0.01	—
Mg	0.30 ± 0.01	—
Mn	0.02 ± 0.01	—
Na	0.40 ± 0.10	—
P	0.42 ± 0.01	—
Pb	5.32 ± 1.19	0.14 – 0.60
S	4.10 ± 0.06	—
V	0.04 ± 0.01	0.13
Zn	0.05 ± 0.01	0.20 – 0.36

[a]Maximum allowed values for soils with residential/parkland, commercial and industrial use, according Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health (CCME, 2007).

Table 3 Characteristics of soils samples after application of hydrophilic polyacrylate polymers and growth of *Spergularia purpurea* (Mean ± SD; *n* = 3).

	Control	Diapers	Polymer from Diapers	Polymer 7015
pH (H_2O)	5.89 ± 0.44[b]	7.00 ± 0.34[a]	6.94 ± 0.25[a]	6.96 ± 0.21[a]
EC (µS/cm)	92.93 ± 24.24[a]	111.99 ± 19.79[a]	135.19 ± 40.53[a]	108.26 ± 25.43[a]
Organic C (g/kg)	2.06 ± 0.08[b]	3.91 ± 0.45[a]	4.43 ± 1.04[a]	3.58 ± 0.52[a]
N (mg/kg)				
Total	201.11 ± 29.56[a]	238.06 ± 30.64[a]	216.13 ± 28.15[a]	201.93 ± 10.58[a]
N-NH_4	92.60 ± 44.11[a]	24.43 ± 8.76[b]	28.20 ± 2.56[b]	20.87 ± 9.36[b]
N-NO_3	104.33 ± 40.79[a]	101.77 ± 16.19[a]	148.30 ± 74.45[a]	114.00 ± 24.38[a]
Extractable P (mg/kg)	1.32 ± 0.38[a]	1.26 ± 0.19[a]	2.12 ± 0.36[a]	1.48 ± 0.48[a]
Extractable K (mg/kg)	39.01 ± 2.49[b]	71.10 ± 32.72[b]	143.04 ± 27.86[a]	82.17 ± 22.84[b]
Fe in iron oxides (g/kg)				
Non crystalline Fe oxides	0.11 ± 0.02[a]	0.10 ± 0.05[a]	0.19 ± 0.12[a]	0.14 ± 0.01[a]
Crystalline Fe oxides	11.73 ± 1.21[a]	13.19 ± 2.72[a]	11.83 ± 1.13[a]	11.92 ± 1.72[a]

Different letters in the same row indicate significant differences ($p < 0.05$).

Extractable K concentration was the highest in the soil amended with the polymer from diapers (Table 3). Although no significant differences were found for total N and N-NO_3 concentrations, N-NH_4 was the highest in control. This suggests that a greater uptake of N took place during growth of S. purpurea, related with a greater biomass accumulation in amended soils (Qu and de Varennes, 2010). The higher concentrations of N-NO_3 and N-NH_4 in the control compared to the initial soil can be related to increased microbial activity in soil as a consequence of plants growth and irrigation.

In general, the rehabilitation using polyacrylate polymers, independently of the source, and *S. purpurea* growth improved the chemical characteristics of this type of soils developed on *gossan* mine wastes.

Extractable soil solutions

The three different extraction solutions which were used in this work represented distinct processes and/or chemical reactions that can occur in soil, simulating the potential availability of several elements, including hazardous elements, for leaving organisms. This comparison will contribute to a better understanding of the behaviour of the polyacrylate polymer in soil during the rehabilitation processes. The amendments increased the pH of leachates and pore water compared to control and initial soil. No significant differences in EC from leachates were observed; however the EC values in pore water were almost double following polymer application (Fig. 1). Leachates and pore waters for the same treatment had similar pH values suggesting that there were few materials capable of generating acidity.

Concentrations of some chemical elements in the leachates (Cu, Mn, Pb, V and Zn), pore water (Al, Cu, Mn, Pb, V and Zn) and RHIZO solution (Cu, Mn, Pb and V) were below the detection limit of the apparatus (mg/L – Al and Pb: 0.25; Cu, Mn and Zn: 0.05; V: 0.5). The increase of soil pH and organic C, due to polymer application, did not influence the concentrations of the elements in these fractions.

The elements in aqueous solutions (leachates and pore water) and RHIZO solution from initial soil represented less than 6.7 % and 8.6 % of their total concentrations, respectively (Figs. 2–4), with the exception of Ca in pore water (18.9–28.4 % of the total) and RHIZO fraction (33.6–50.4 % of the total). These percentages are slightly higher than those in the aqueous solutions extracted with the rhizosphere based-method, in some soils from São Domingos, Caveira and Chança mining areas from IPB (Abreu et al., 2012a,b). Similar observations were made during the experiment, as these fractions in all the treatments (control and amended) were in the same range compared to those in initial soil, except for Na (Leachates: 11.5–32.9 % of total concentration; Pore water: 7.8–60.3 % of total concentration). This exception may be related to the addition of artificial urine (as described in Material and Methods) and the presence of Na+ as counter ion in the polymer. Although the soil had large total concentrations of some elements (like As, Cu and Pb), the fractions potentially available for organisms, before and after polymer application, were small and may represent a low potential environmental risk.

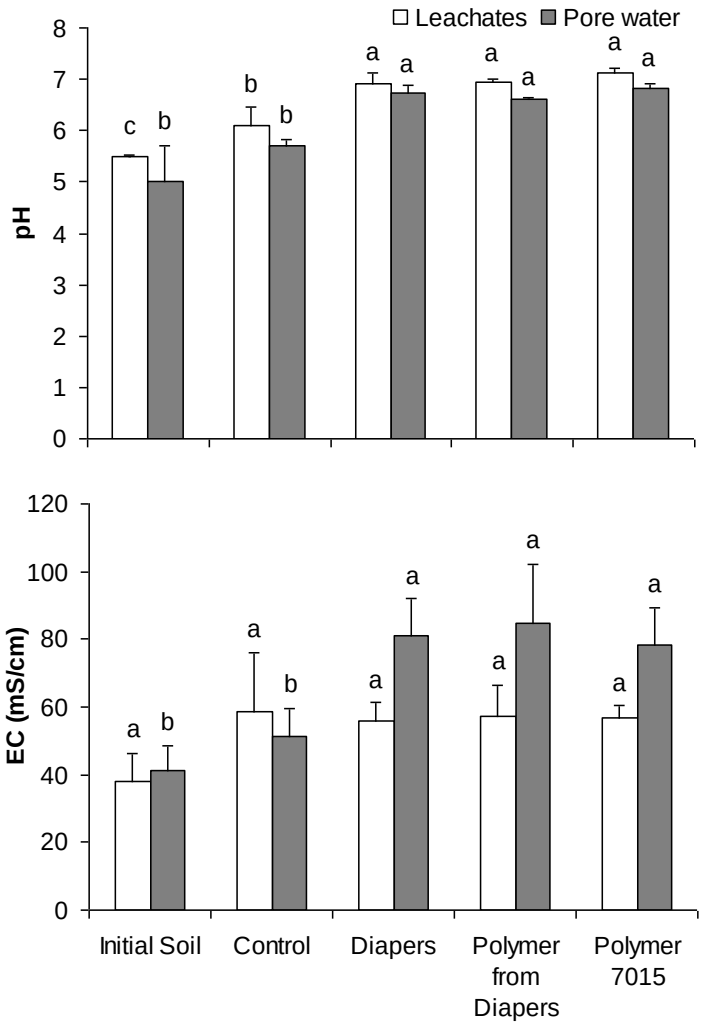

Fig. 1 Values of pH and electrical conductivity (EC) in simulated leachates (DIN extraction) and pore water solution from initial soil and soils from treatments of the mesocosm assay (control; soils amended with diapers, polymer from diapers and synthetic polymer 7015). Different letters in the same parameter and extractable solution indicate significant differences ($p < 0.05$).

The concentrations of chloride and nitrate in the leachates and soil pore water, Mg concentrations in leachates, as well as Zn concentrations in RHIZO solution did not present significant differences (Figs. 2–4).

The NH_4^+ from urine in control may have promoted Al exchange from the soil to the leachate, compared to the initial soil. However, independently of the source, polymer application led to aretention of Al ions on solid phases, reducing its concentration in leachates (Fig. 2).

The low concentrations of Ca, K and sulfate in leachates from control and amended treatments, compared to initial soil leachates, can be related to soil reaction and their leaching as a consequence of rain and irrigation during the mesocosm experiment (Figs. 2 and 3).

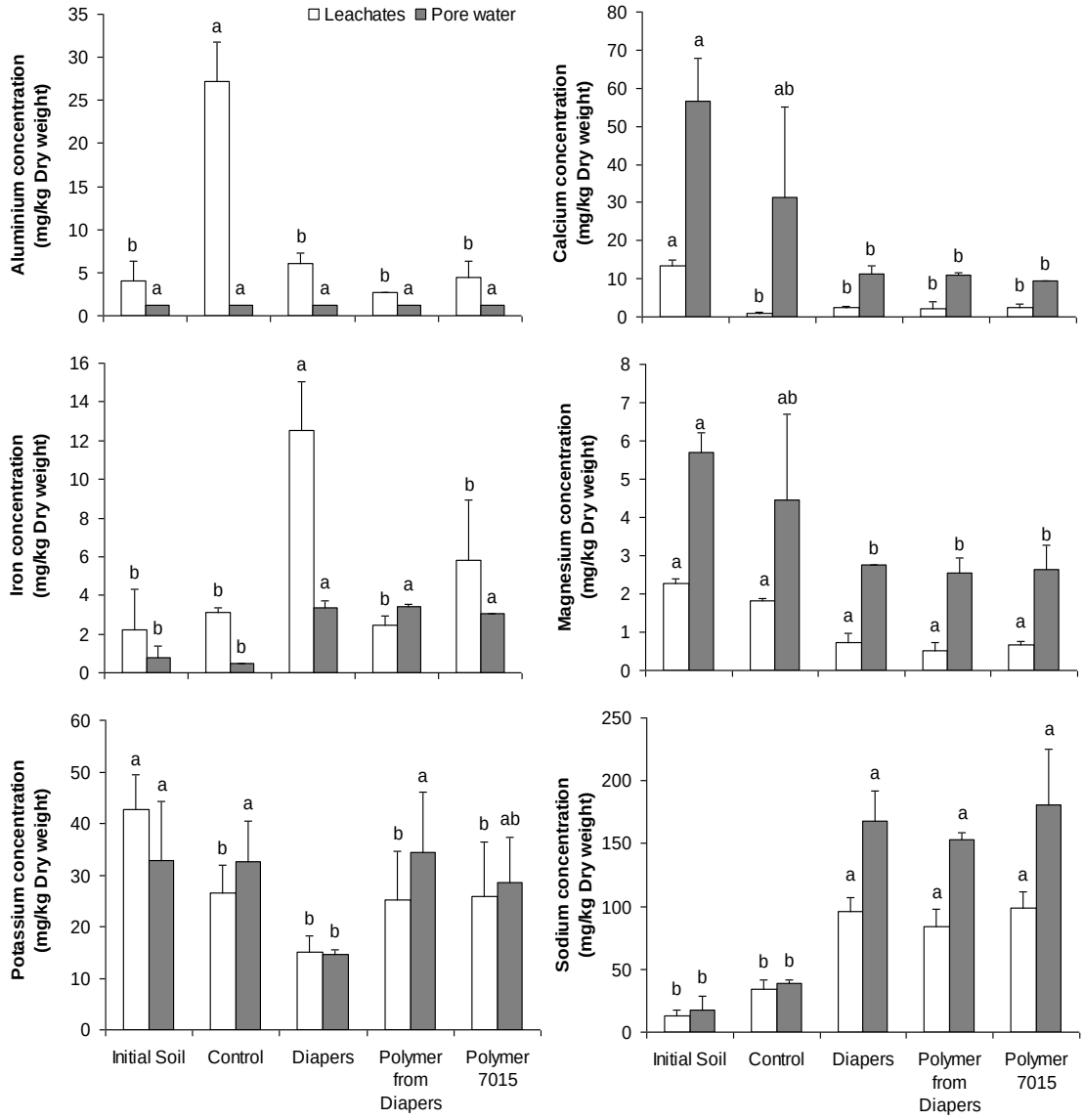

Fig. 2 Cation concentrations in simulated leachates (DIN extraction) and pore water solution from initial soil and soils from treatments of the mesocosm assay (control; soils amended with diapers, polymer from diapers and synthetic polymer 7015). Different letters in the same parameter and extractable solution indicate significant differences ($p < 0.05$).

The materials present in diapers seemed to influence the concentration of Fe in leachates, as the highest values in leachates (12.50 mg/kg; Fig. 2) were observed in this treatment.

Amended soils had the highest concentrations of As, Na and phosphate in leachates and pore water (Figs. 2 and 3). The high As concentration in aqueous solutions may be explained by the negative charges on the polyacrylates reactive groups promoting some cation adsorption, inhibiting the formation of low solubility solid phases (*e.g.* metal arsenates; Santos et al., 2012). The exchange between counter ions (Na^+) in the polymers and acidic ions in the soil can explain the higher Na concentration. The increase of the phosphatase activity in amended soil after S. purpurea growth (Qu and de Varennes, 2010) probably promoted a higher release of phosphate ions.

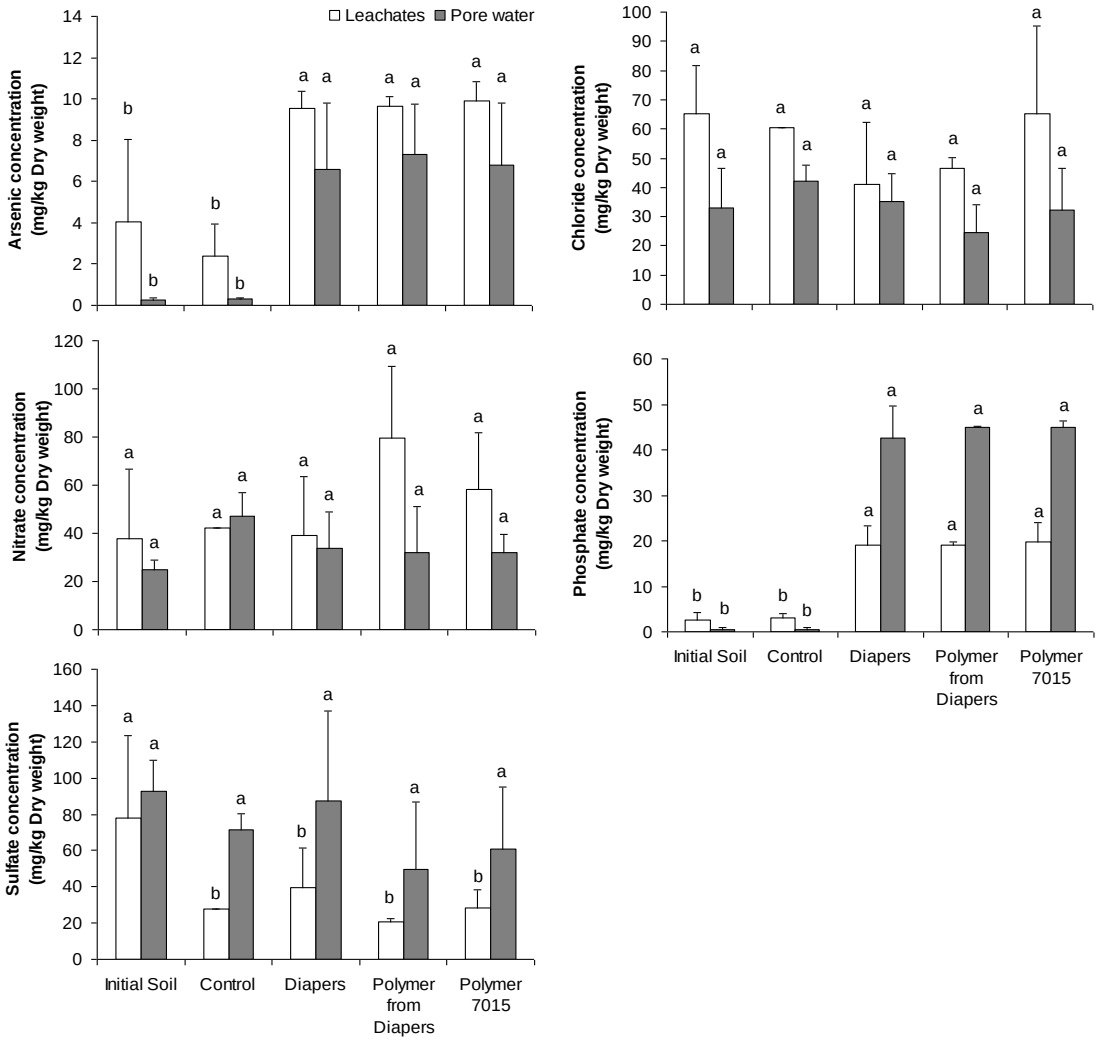

Fig. 3 Anions concentrations in simulated leachates (DIN extraction) and pore water solution from initial soil and soils from treatments of the mesocosm assay (control; soils amended with diapers, polymer from diapers and synthetic polymer 7015). Different letters in the same parameter and extractable solution indicate significant differences ($p < 0.05$).

Differences between concentrations of As, Fe and K in RHIZO solution from the treatments amended with diapers and amended with only polymer (from diapers or polymer 7510) were observed (Fig. 4). Polymer application increased the concentration of elements in RHIZO solution, except for Zn (Fig. 4). This can also be associated with the greater *S. purpurea* growth in these treatments (Qu and de Varennes, 2010) and more exudation of low-molecular-weight organic acids by roots, capable of forming complexes and enhancing the availability of the chemical elements (Feng et al., 2005).

Comparing results from leachates and pore water (Figs. 2 and 3), the concentrations of Ca and sulfate in all treatments, as well as Mg, Na and phosphate in amended treatments increased more than 20 % in pore water fraction with a simultaneous increase of the EC. Thus, the longer contact with water seems to promote the dissolution of solid phases containing these elements and exchanges with ions in the polymer. Moreover, Al and As were reduced drastically (24–99 %), as well as Fe in the majority of the treatments, in pore water compared to leachates (Figs. 2 and 3), suggesting the formation of new solid phases containing these chemical elements.

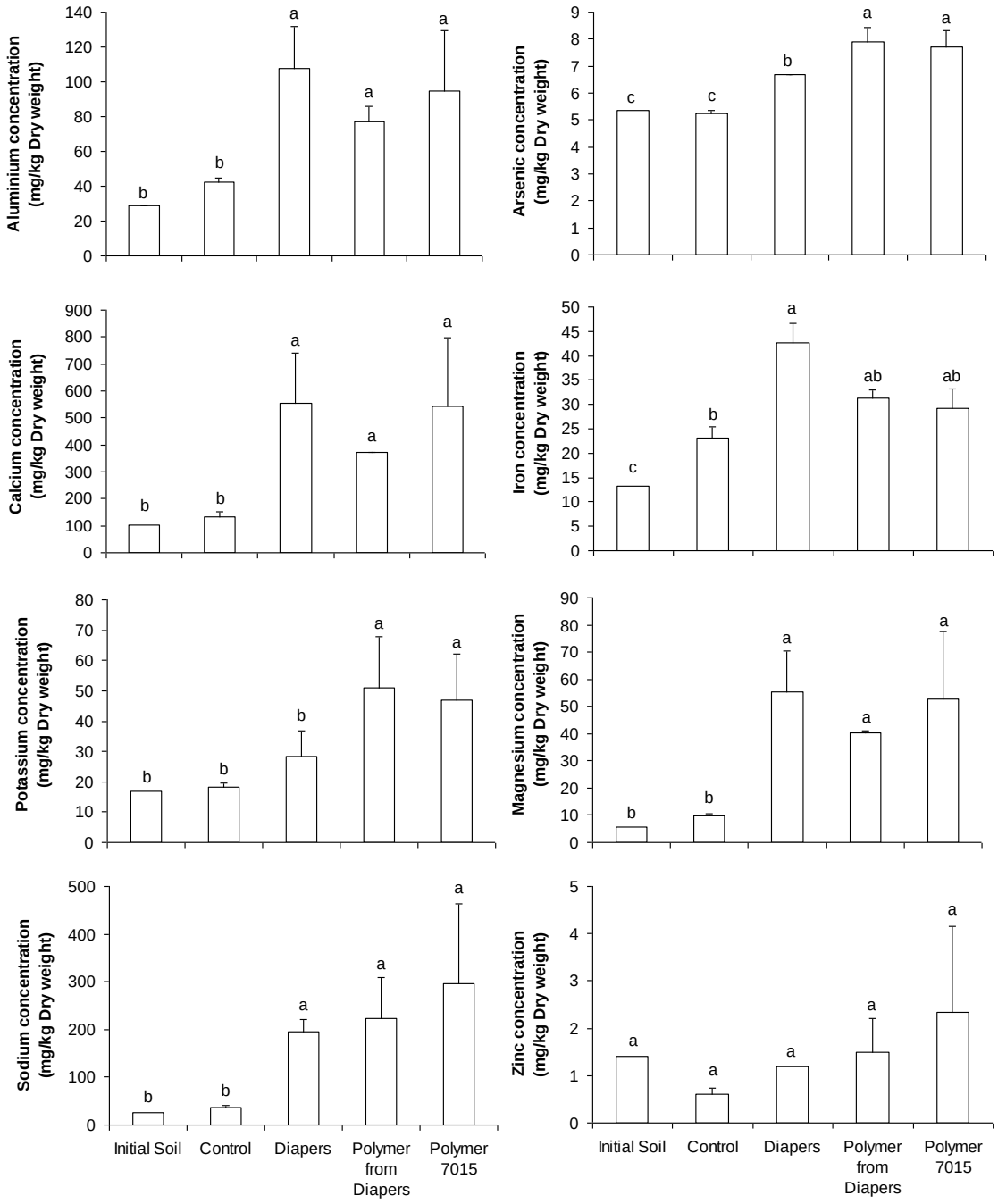

Fig. 4 Chemical elements concentrations in simulated rizosphere solution, extracted with an organic acid solution, from initial soil and soils from treatments of the mesocosm assay (control; soils amended with diapers, polymer from diapers and synthetic polymer 7015). Different letters indicate significant differences ($p < 0.05$).

In general, the polymer matrix seems to retain some essential cations, like Ca, Fe, K and Mg, and hazardous elements, like Al, influencing their availability in soil. However, this behaviour was not clearly observed in the leachates and pore water. The equilibria established between water:soil:polymer, even in the pore water, were not enough to evaluate the total amounts of the elements retained in the polymer as water can only extract the chemical elements weakly adsorbed. The RHIZO solution, due to the presence of organic acids, is a stronger extractant than water. That

solution simulates the rhizosphere processes, namely the complexation of elements released from inorganic and organic colloids exchangeable positions and, in the present assays, also from the polyacrylate polymer. In fact, concentrations of the elements in RHIZO solution were higher than those in leachates and pore water (except for As, K and Na).Nevertheless, this fact suggests that the polymer matrix can retain a large amount of cations, which can be uptake by the plants only through root exudation of organic acids. The increase of the available fraction of the nutrients by the rizosphere effect can be an advantage for soil rehabilitation processes.

Although some hazardous elements increased in the fractions potentially available for organisms after polymer application, their concentrations remained low and with small environmental risk.

Plant development in the lysimeters

Spontaneous species belonging to the Poaceae, Fabaceae and Asteraceae families (common in the mining area), were identified but as expected, *S. purpurea* was the dominant species, with a considerable biomass from November until June. At the end of the experiment, the fresh biomass of plants varied with treatment reaching the highest values in control (134.5 g) and in soil amended with polymer from diapers (131.7 g), compared to soil amended with the 7015 polymer or shredded diapers (77.7 and 71.4 g, respectively). However, a clear distinction between the chemical characteristics of soils from these two groups was not observed. The great heterogeneity of plants species and inexistence of a considerable quantity of a representative species in some periods did not provide a clear tendency of natural colonization in relation to soil treatments.

In the first month of the experiment, the highest germination rate for *C. ladanifer* was in control (22 seedlings) but for *L. sampaioana* it was in the treatment with the 7015 polymer (20 seedlings). However, even in amended soil the mortality rate was very high (over 80 %) and very few shrubs survived, which was the opposite of the observed with *S. purpurea* (Qu and de Varennes, 2010).

Plants bioassays

Plant characteristics (*e.g.* seed properties, root morphology and physiology and tolerance/ sensitiveness) can modify the plant response to toxicity of leachates or contaminated soils (Sheppard et al., 1993). In the present experiment, visible symptoms of toxicity were observed in lettuces in initial and amended soil using the filter paper test (leaves with light greenish-yellow colour) and in oats from amended treatments using the hydroponic test (leaves with dry tips or brown coloration).

Growth of *Brassica rapa* was also very sensitive to soil physical and chemical properties in the bioassays (Sheppard et al., 1993). Bioassays using leachates or water soil extracts can predict the risks of contaminants in ground and surface waters, but they do not show interactions and exchanges that occur in soils (Leitgib et al., 2007; van Gestel et al., 2001). Therefore, ecotoxicological tests, combining both direct (with whole soil) and indirect (with leachate) are useful.

No differences were observed among treatments in the studied parameters when using lettuce in filter paper and soil tests (Table 4). However, with oat most parameters were different between

treatments (Table 5). The seed germination test using both species was low sensitive what can be related to the low concentration of elements in leachates. Similar results were obtained for germination of lettuce and other species (broccoli, tomato and radish) with aqueous solutions of Pb or Cu (Salvatore et al., 2008), and lettuce, both with leachates from soils affected by the Aznalcóllar pyrite-mine spill (Valerio et al., 2007) and saturation extracts from sediments collected in the Rodalquilar mining district (Bagur-González et al., 2011). In addition, Salvatore et al. (2008) also pointed out that seed coats act as a barrier which protects the growth of the embryo. Although root growth is known to be more sensitive than germination to chemical elements toxicity (Araùjo and Monteiro, 2005; Martí et al., 2007; Salvatore et al. 2008; Valerio et al., 2007), the concentrations of elements in leachates and RHIZO solution of the soils were too low (Figs. 2–4) to inhibit the growth of lettuce roots. In contrast, oat root elongation in the treatment with polymer from diapers was the lowest in the filter paper test, although the chemical characteristics of leachates from this treatment were not different compared to the other amended treatments (Figs. 2 and 3). Furthermore, oat root elongation was not correlated to the studied chemical characteristics of the leachates.

In the soil test, the increase of organic C and N in control (Tables 1 and 3), compared with initial soil, seems to have promoted oat growth. Initial soil (and control) showed low toxicity as reported for other *gossan* samples from São Domingos by Alvarenga et al. (2012).

In the soil test, the oat root and shoot elongations (Table 5) were negatively influenced by the polymer application due to some toxicity of this polymer or high concentrations of As, Fe and Na in pore water ($-0.83 < r < -0.95$ depending on element). Moreover, in amended treatments the increase of As and Mg in the RHIZO solution (Fig. 4) could have contributed to reduce shoot elongation (r = –0.89 and –0.82, respectively). Nevertheless, the coexistence of high concentrations of various chemical elements in the RHIZO solution (Al, As, Ca, Fe, Mg and Na) or pore water (As, Fe and Na) from amended treatments, compared to the control, may have influenced plant growth by direct or/and antagonism/synergism processes during uptake and translocation.

In the hydroponic bioassay, the treatment amended with diapers presented the lowest lettuce shoot elongation (Table 4), but the fresh biomass presented one of the highest values (together with the treatment amended with polymer from diapers). The high total fresh biomasses were not related to the increase of water accumulation in tissues (data not shown). Although the concentration of Fe in leachates (the only element which varied between treatments; Figs. 2 and 3) did not influence negatively and directly shoot elongation, antagonism or synergism behaviours could occur and influence the translocation of other elements, having significant consequences for plant growth (Kabata-Pendias, 2011). In general, the studied chemical characteristics of leachates were not different between amended treatments suggesting that the variation in fresh biomass was related to other properties that were not analysed. For lettuce (Table 4), all amended treatments presented higher values of root elongation compared to control and initial soil, probably due to the higher phosphate concentration in leachates (r = 0.95). In contrast, the increase of As concentration in leachates from amended treatments showed no influence in the root elongations (r = 0.88).

Table 4 Biological parameters obtained in the bioassays with *Lactuca sativa* (Mean ± SD; *n* = 3).

	Initial soil	Control	Diapers	Polymer from Diapers	Polymer 7015
			Filter paper test		
Seeds germination (%)	95 ± 7[a]	90 ± 16[a]	81 ± 22[a]	90 ± 8[a]	86 ± 14[a]
Aerial part elongation (mm)	4 ± 1[a]	6 ± 1[a]	5 ± 1[a]	7 ± 1[a]	10 ± 6[a]
Root elongation (mm)	20 ± 12[a]	20 ± 8[a]	18 ± 2[a]	33 ± 11[a]	48 ± 35[a]
Fresh biomass (mg)	41.1 ± 10.3[b]	58.5 ± 10.1[ab]	52.4 ± 25.0[ab]	73.1 ± 14.4[a]	67.7 ± 11.3[ab]
			Soil test		
Seeds germination (%)	57 ± 29[a]	81 ± 8[a]	52 ± 22[a]	38 ± 16[a]	57 ± 25[a]
Aerial part elongation (mm)	18 ± 4[a]	16 ± 3[a]	18 ± 11[a]	18 ± 4[a]	19 ± 2[a]
Root elongation (mm)	35 ± 10[a]	36 ± 2[a]	36 ± 12[a]	47 ± 38[a]	19 ± 10[a]
Fresh biomass (mg)	0.07 ± 0.03[a]	98.2 ± 28.4[a]	57.4 ± 60.1[a]	29.8 ± 15.7[a]	40.9 ± 12.2[a]
			Hydroponic test		
Aerial part elongation (mm)	25 ± 1[a]	25 ± 2[a]	19 ± 1[b]	25 ± 3[a]	23 ± 3[a]
Root elongation (mm)	64 ± 9[b]	58 ± 9[b]	80 ± 7[a]	81 ± 11[a]	92 ± 12[a]
Fresh biomass (mg)	196.2 ± 15.5[b]	196.7 ± 9.2[b]	230.4 ± 22.2[a]	244.1 ± 1.0[a]	179.6 ± 20.3[b]

Different letters in same row indicate significant differences ($p < 0.05$).

Table 5 Biological parameters obtained in the bioassays with *Avena sativa* (Mean ± SD; *n* = 3).

	Initial soil	Control	Diaper	Polymer from Diaper	Polymer 7015
			Filter paper test		
Seeds germination (%)	100 ± 0[a]	73 ± 12[a]	47 ± 50[a]	73 ± 31[a]	100 ± 0[a]
Aerial part elongation (cm)	11.3 ± 1.6[a]	14.3 ± 0.9[a]	13.8 ± 0.7[a]	11.2 ± 2.5[a]	14.0 ± 0.8[a]
Root elongation (cm)	8.6 ± 0.9[ab]	9.6 ± 1.0[a]	9.7 ± 0.3[a]	6.9 ± 1.8[b]	8.7 ± 0.2[ab]
Fresh biomass (mg)	501.7 ± 83.1[a]	495.2 ± 87.8[a]	437.8 ± 139.1[a]	416.0 ± 194.7[a]	602.7 ± 112.4[a]
			Soil test		
Seeds germination (%)	7 ± 12[b]	13 ± 23[ab]	20 ± 20[ab]	47 ± 12[a]	20 ± 20[ab]
Aerial part elongation (cm)	17.0 ± 0.1[a]	16.9 ± 0.9[a]	11.5 ± 1.2[b]	11.1 ± 2.0[b]	11.5 ± 0.1[b]
Root elongation (cm)	7.4 ± 0.1[a]	6.9 ± 1.7[a]	1.4 ± 0.3[c]	1.9 ± 0.5[c]	4.4 ± 2.0[b]
Fresh biomass (mg)	118.9 ± 1.0[b]	329.9 ± 1.0[a]	232.5 ± 167.6[ab]	287.0 ± 42.9[a]	193.8 ± 128.0[ab]
			Hydroponic test		
Aerial part elongation (cm)	15.3 ± 1.8[a]	14.5 ± 2.7[a]	8.4 ± 2.1[b]	7.0 ± 2.7[b]	9.9 ± 1.7[b]
Root elongation (cm)	6.5 ± 0.4[ab]	7.2 ± 0.9[a]	5.3 ± 1.2[ab]	4.5 ± 1.5[b]	5.8 ± 0.9[ab]
Fresh biomass (mg)	879.8 ± 154.5[a]	667.1 ± 119.4[ab]	478.2 ± 83.6[b]	313.2 ± 1.0[b]	417.6 ± 173.1[b]

Different letters in same row indicate significant differences ($p < 0.05$).

Comparing the toxicity indexes reported by Bagur-González et al. (2011), that used paper filter test and distilled water as blank, the leachates from all the present treatments and initial soil indicated no (indexes values > 1) or low (indexes values from 0 to –0.25) toxicity depending on plant parameter and plant species. Lettuce seed germination was less sensitive (germination index of all treatments: –0.05 to –0.15) than oat (germination index values > 0, except for the treatment amended with diapers that presented an index value = –0.25). In general, indexes of shoot and root elongations for both plant species did not suggest a large toxicity (never more severe than low toxicity). For roots, this fact may be due to the low elements concentrations, while for shoots the potential toxicity also depends on elements translocation.

For evaluation of the toxicity in aerial part and root elongations, the hydroponic test seemed more appropriate to evaluate toxicity but the determination of root length from the assays based on plant growth can be more sensitive due to the direct influence of the elements from the leachates.

Microalgae and microcrustacean bioassays

All bioassays were valid according to the criteria established in the guidelines. No significant differences in the responses of microalgae and Daphnia were observed between leachates from initial and control soils: around 97 % of toxicity effects on *D. magna* (Fig. 5) and stimulation of algae growth (inhibition effect: –25 % I and –12 % I, respectively; Fig. 6) suggests that the growth cycle of *S. purpurea* did not decrease the toxicity of leachates towards these organisms. Initial soil and control had a considerable toxicity only when the indirect test with *D. magna* was used. Similar results were obtained in leachates from soils collected in Caveira and Lousal mines which showed 100 % of *D. magna* mortality but percentages of *P. subcapitata* growth inhibition null for Lousal soils and between 22 and 87 % for Caveira soils (Rocha et al., 2011). Other authors also reported the existence of toxicity in other *gossan* samples from São Domingos (EC50 of 26 % *V*/*V*; Alvarenga et al., 2012) and Aljustrel mine soils (EC50 of 36 % *V*/*V*; Alvarenga et al., 2008) using the D. magna test.

In the D. magna bioassay (Fig. 5), the leachates from control and treatment amended with diapers presented the highest acute toxicity values (98 % and 90 % of toxic effect, respectively). However, the inhibition of microalgae growth occurred in the treatments associated with the use of diapers (Fig. 6; Diapers: 6 % I; Polymer from diapers: 27 % I) suggesting a negative influence of the polymer from the diapers, which may be different from polymer 7015. No chemical characteristic of the leachates was strongly correlated with toxicity for daphnia or microalgae. However, the coexistence of several chemical elements, even at low concentrations, may amplify acute toxicity (Holmstrup et al., 2010).

The microalgae bioassay presented a large variability between replicates which may be related to the *gossan* heterogeneity (Santos et al., 2012), and consequently the chemical characteristics of the leachates (e.g. contents of organic C and nutrients). The growth of microalgae can increase in the presence of dissolved organic ligands and/or high hydrophobic dissolved organic matter to Fe(III) ratio (Lee et al., 2009). Thus, the microalgae bioassay seems to be less sensitive when evaluating ecotoxicity of leachates with low concentrations of toxic elements and adequate contents of nutrients and organic C. The *D. magna* bioassay showed a clear sensitivity for the leachates with low elements

concentrations. These results agree with those reported by Sheppard et al. (1993). The application of the synthetic polymer suppressed soil toxicity to both studied organisms.

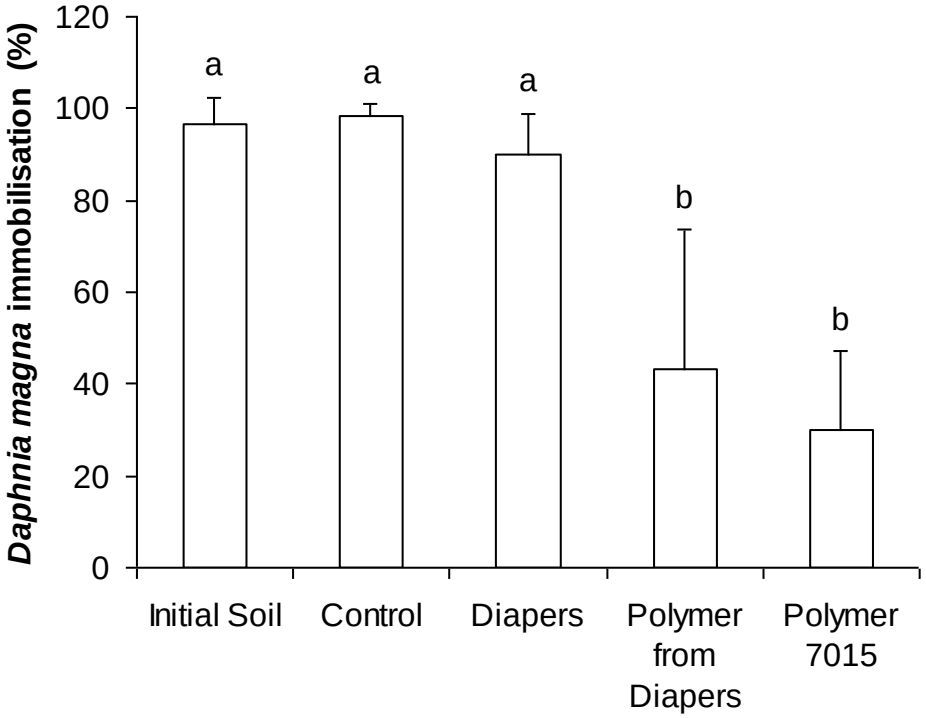

Fig. 5 Percentage of immobilisation of *Dapnhia magna* - acute toxicity test of soil leachates from initial soil and soils from treatments of the mesocosm assay (control; soils amended with diapers, polymer from diapers and synthetic polymer 7015). Different letters indicate significant differences ($p < 0.05$).

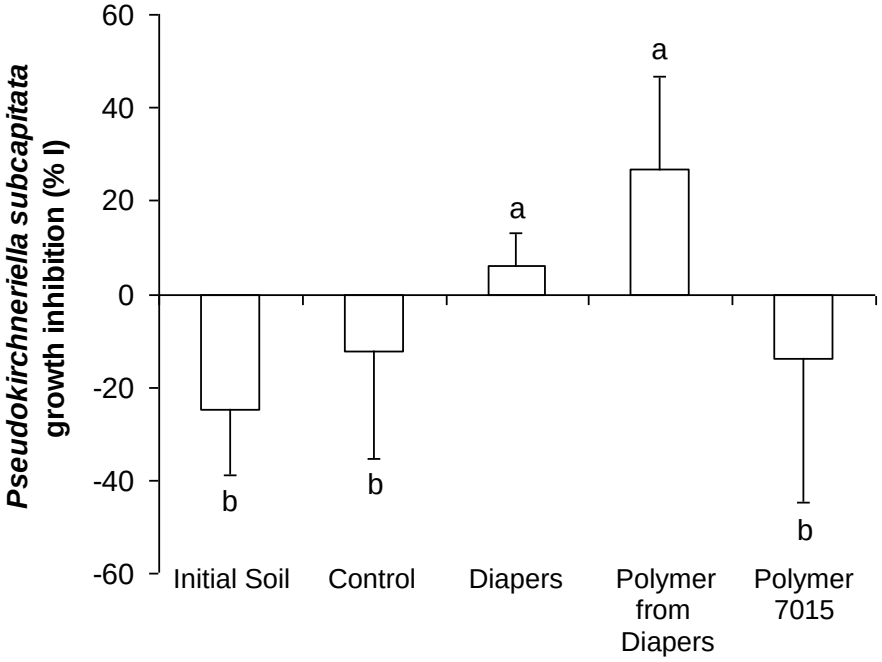

Fig. 6 Growth inhibition percentage of microalgae - toxicity test with soil leachates from initial soil and soils from treatments of the mesocosm assay (control; soils amended with diapers, polymer from diapers and synthetic polymer 7015). Different letters indicate significant differences ($p < 0.05$).

CONCLUSIONS

Despite the high total concentrations of several elements (Al, As, Cu, Pb) in the soil developed on *gossan*, the percentage of elements in soluble fractions (evaluated by the simulated leachates and pore water) and in the rizosphere conditions (evaluated by the RHIZO solution) were very small. Bioassays using different organisms (monocot and dicot species, microcrustacean and microalgae) showed the low toxicity of the soil and leachates, and the low environmental risk they pose. The low toxicity of the soil is due to the low concentrations of the chemical elements in the fraction usually referred as available (extracted with several aqueous solutions) for organisms, which were not negatively influenced.

Daphnia magna test was the only test that showed a clear difference of toxicity between the control and amended soils leachates. The different sensitiveness of bioassays shows that several ecotoxicological tests should be used to assess environmental risk of soil contamination and its rehabilitation

Growth of *S. purpurea* and natural plant colonization improved chemical soil characteristics, but the use of the polyacrylate polymers contribute to a further improvement in soil quality (pH and fertility). However, for both autochthones shrubs, *C. ladanifer* and *L. sampaioana*, the improvement in soil conditions did not promote germination and development of the majority of seedlings.

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REFERENCES

Abreu, M.M., Batista, M.J., Magalhães, M.C.F., Matos, J.X., 2010a. Acid mine drainage in the Portuguese Iberian Pyrite Belt. In: Brock C.R. (Eds.), Mine drainage and related problems. Nova Science Publishers, Inc. New York, pp. 71-118.

Abreu, M.M., Santos, E.S., Ferreira, M., Magalhães, M.C.F., 2012a. *Cistus salviifolius* a promising species for mine wastes remediation. Journal of Geochemical Exploration 113, 86-93.

Abreu, M.M., Santos, E.S., Fernandes, E., Magalhães M.C.F. 2012b. Trace elements tolerance, accumulation and translocation in *Cistus populifolius*, *Cistus salviifolius* and their hybrid growing in polymetallic contaminated mine areas. Journal of Geochemical Exploration 123, 52-60.

Activation Laboratories, 2012. 1H - Total Digestion - ICP, INAA. Available in: www.actlabs.com/page.aspx?page=506&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes2010 (Accessed May 2012)

Adriano, D.C., 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability and risk of metals. New York, Springer-Verlag.

Al-Humaid, A.I., Moftah, A.E., 2007. Effects of hydrophilic polymers on the survival of buttonwood seedlings grown under drought stress. Journal of Plant Nutrition 30, 53-66.

Alvarenga, P., Palma, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008b. Evaluation of tests to assess the quality of mine-contaminated soils. Environmental Geochemistry and Health 30, 95-99.

Alvarenga, P., Palma, P., de Varennes, A., Cunha-Queda, A.C., 2012. A contribution towards the risk assessment of soils from the São Domingos Mine (Portugal): chemical, microbial and ecotoxicological indicators. Environmental Pollution 161, 50-56.

Álvarez-Valero, A.M., Pérez-López, R., Matos, J., Capitán, M.A., Nieto, J.M., Sáez, R., Delgado, J., Caraballo, M., 2008. Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian Peninsula): evidence from a chemical and mineralogical characterization. Environmental Geology 55(8), 1797-1809.

Araùjo, A.S.F., Monteiro, R.T.R., 2005. Plant bioassays to assess toxicity of textile sludge compost. Scientia Agricola 62, 286-290.

Boatright, J.L., Balint, D.E., Mackay, W.A., Zajicek, J.M., 1997.Incorporation of a hydrophilic polymer into annual landscape beds. Journal of Environmental Horticulture 15, 37-40.

Bagur-González, M.G., Estepa-Molina, C., Martín-Peinado, F., Morales-Ruano, S. 2011. Toxicity assessment using *Lactuca sativa* L. bioassay of the metal(loid)s As, Cu, Mn, Pb and Zn in soluble-in-water saturated soil extracts from an abandoned mining site. Journal of Soils and Sediments 11(2), 281-289.

Buján, E., Garcia-Arrese, A., Velasco-Molina, M., Macías, F., 2010. Soil solution in soils under *Erica andevalensis* formations in the Riotinto mining area (Huelva, SW Spain). Revista de Ciências Agrárias 33(1), 111-118.

CCME – Canada Council of Ministers of the Environment. Canadian soil quality guidelines for the protection of environmental and human health: Summary tables (updated September, 2007). Winnipeg: CCME; 2007.

Cerejeira, M.J., Pereira, T., Silva-Fernandes, A., 1998. Use of new microbiotests with *Daphnia magna* and *Selenastrum capricornutum* immobilized forms. Chemosphere 37(14-15), 2949-2955.

Corral, R., Pita, J.M., Pérez-García, F. 1990. Some aspects of seed germination in four species of *Cistus* L.. Seed Science and Technology 18 321-325.

de Endredy, A.S., 1963. Estimation of free iron oxides in soils and clays by photolytic method. Clay Minerals Bulletin 9, 209-217.

de Varennes, A., Torres, M.O., Conceição, E., Vasconcelos, E., 1999. Effect of polyacrylate polymers with different counter ions on the growth and mineral composition of perennial ryegrass. Journal of Plant Nutrition 22, 33-43.

DIN 38414-S4, 1984. Schlamm und Sedimente, Bestimmung der Eluierbarkeit mit Wasser. DIN Deutsches Institut für Normung, Berlin.

Feng, M., Shan, X., Zhang, S., Wen, B., 2005. A comparasion of rizosphere-based method with DTPA, EDTA, $CaCl_2$ and $NaNO_3$ extraction methods for prediction of bioavailability of metals in soil to barley. Environmental Pollution 137, 231-240.

Ferrari, B., Radetski, C.M., Veber, A.M., Ferard, J.F., 1999. Ecotoxicological assessment of solid wastes: a combined liquid- and solid-phase testing approach using a battery of bioassays and biomarkers. Environmental Toxicology and Chemistry 18(6), 1195-1202.

García-Lorenzo, M.L., Martínez-Sánchez, M.J., Pérez-Sirvent, C., Molina, J., 2009. Ecotoxicological evaluation for the screening of areas polluted by mining activities. Ecotoxicology 18, 1077-1086.

Guiwei, Q., de Varennes, A., Cunha-Queda, C. 2008. Remediation of a mine soil with insoluble polyacrylate polymers enhances soil quality and plant growth. Soil Use and Management 24, 350-356.

Holmstrup, M., Bindesbøl, A.M., Oostingh, G.J., Duschl, A., Scheil, V., Köhler, H.R., Loureiro, S., Soares, A.M.V.M., Ferreira, A.L.G., Kienle, C., Gerhardt, A., Laskowski, R., Kramarz, P.E., Bayley, M., Svendsen, C.,

Spurgeon, D.J., 2010. Interactions between effects of environmental chemicals and natural stressors: A review. Science of the Total Environment 408, 3746-3762.

ISO 8692, 2004. Water Quality – Freshwater algal growth inhibition test with unicellular green algae. International Standard Organization.

ISO 6341, 1996. Water quality – Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) – Acute toxicity test. International Standard Organization.

IUSS Working Group WRB, 2007. World Reference Base for Soil Resources 2006, first update 2007. World Soil Resources Reports No. 103. Rome: FAO.

Kabata-Pendias, A., 2011. Trace elements in soils and plants. 4th ed. Boca Raton: CRC Press.

Kabata-Pendias, A. 2004. Soil-plant transfer of trace elements-an environmental issue. Geoderma 122, 143-149.

Knoke, K.L., Marwood, T.M., Cassidy, M.B., Liu, D., Seech, A.G., Lee, H., Trevors, J.T., 1999. A comparison of five bioassays to monitor toxicity during bioremediation of pentachlorophenol-contaminated soil. Water, Air and Soil Pollution 110, 157-169.

Lee, J., Park, J.H., Shin, Y.S., Lee, B.C., Chang, N.I., Cho, J., Kim, S.D., 2009. Effect of dissolved organic matter on the growth of algae, *Pseudokirchneriella subcapitata*, in Korean lakes: The importance of complexation reactions. Ecotoxicology and Environmental Safety 72, 335-343.

Leitgib, L., Kálmán, J., Gruiz, K., 2007. Comparison of bioassays by testing whole soil and their water extract from contaminated sites. Chemosphere 66, 428-434.

Macías, F., 2004. Recuperación de suelos degradados, reutilización de resíduos y secuestro de carbono. Una alternativa integral de mejora de la calidad ambiental. Recursos Rurais 1, 49-56.

Matos, J.X., Martins, L.P., 2006. Reabilitação ambiental de áreas mineiras do sector português da Faixa Piritosa Ibérica: estado da arte e perspectivas futuras. Boletín Geológico y Minero 117, 289-304.

Martí, E., Sierra, J., Sánchez, M., Cruañas, R., Garau, M.A., 2007. Ecotoxicological tests assessment of soils polluted by chromium (VI) or pentachlorophenol. Science of the Total Environment 378, 53-57.

MicroBioTests, 2004. AlgalToxKit FTM – Freshwater toxicity test with microalgae. Standard operational procedure. Nazareth, Belgium: MicroBioTests Inc.

MicroBioTests, 2000. DaphToxKitTM – Freshwater toxicity screening test.Standard operational procedure. Nazareth, Belgium: MicroBioTests Inc.

Mulvarey, R.L., 1996. Chemical methods: nitrogen-inorganic forms. In: Sparks, D.L. (Ed.), Methods of soil analysis: Part 3 – Chemical methods. SSSA Book series nº 5.Soil Science Society of America, pp. 1123-1184.

OECD – Organisation for Economic Co-operation and Development, 1984. Alga growth inhibition test.Guidelines for the testing of chemicals. Test guideline 201. Paris: OECD.

OECD – Organisation for Economic Co-operation and Development, 2006. Terrestrial plant test: seedling emergence and seedling growth. Guidelines for the testing of chemicals. Test guideline 208. Paris: OECD.

Pereira, T., Cerejeira, M.J., Brito, F., Morbey, M.A., 2000. Laboratory studies with microbiotests to evaluate the acute toxicity to aquatic biota of herbicides used in Portuguese paddy fields. In: Persoone, G., Janssen, C., De Coen, W. (Eds.), New Microbiotests for routine toxicity screening and biomonitoring. Kluwer London: Academic/Plenum Publishers, pp. 495-500.

Póvoas, I., Barral, M.F., 1992. Métodos de análise de solos. Comunicações do Instituto de Investigação Científica Tropical. Lisboa: Serie Ciências Agrárias 10.

Qu, G., de Varennes, A., 2010. Use of hydrophilic polymers from diapers to aid the establishment of *Spergularia purpurea* in a mine soil. Journal of Hazardous Materials 178, 956-962.

Quental, L., Bourguignon, A., Sousa, A.J., Batista, M.J., Brito, M.G., Tavares, T., Abreu, M.M., Vairinho, M., Cottard, F., 2002. MINEO Southern Europe environment test site. Contamination impact mapping and

modelling - Final Report. Assessing and monitoring the environmental impact of mining activities in Europe using advanced Earth Observation Techniques (MINEO) 5yth FP-IST-1999-10337.

Rocha, L., Rodrigues, S.M., Lopes, I., Soares, A.M.V.M., Duarte, A.C., Pereira, E., 2011. The water-soluble fraction of potentially toxic elements in contaminated soils: Relationships between ecotoxicity, solubility and geochemical reactivity. Chemosphere 84, 1495-1505.

Salvatore, M.D., Carafa, A.M., Carratù, G., 2008. Assessment of heavy metals phytotoxicity using seed germination and root elongation tests: A comparison of two growth substrates. Chemosphere 73, 1461-1464.

Santos, E.S., Abreu, M.M., Nabais, C., Magalhães, M.C.F., 2012. Trace element distribution in soils developed on *gossan* mine wastes and *Cistus ladanifer* L. tolerance and bioaccumulation. Journal of Geochemical Exploration 123, 45-51.

Schwertmann, U., 1964. Differenzierung der eisenoxide des bodens durch extraktion mit ammoniumoxalat-Lösung. Z Planzener Düng Bodenkd 105(3), 194-202.

Sheppard, S.C., Evenden, W.G., Abboud, S.A., Stephenson, M. 1993. A plant life-cycle bioassay for contaminated soil, with comparison to other bioassays: mercury and zinc. Archives of Environmental Contamination and Toxicology 25, 27-35.

Silberbush, M., Adar, E., de Malach, Y., 1993. Use of a hydrophilic polymer to improve water storage and availability to crops grown in sand dunes. I. Corn irrigated by trickling. Agricultural Water Management 23 303-313.

Silva, E., Pereira, A.C., Estalagem, S.P., Moreira-Santos, M., Ribeiro, R., Cerejeira, M.J., 2012. Assessing the quality of freshwaters in a protected area within the Tagus river basin district (central Portugal). Journal of Environmental Quality 41, 1413-1426.

Valerio, M.E., García, J.F., Peinado, F.M., 2007. Determination of phytotoxicity of soluble elements in soils, based on a bioassay with lettuce (*Lactuca sativa* L.). Science of the Total Environment 378, 63-66.

USEPA – United States Environmental Protection Agency 1979. Methods for chemical analysis of water and waste. Cincinatti: Office of Research and Development, US Environmental Protection Agency, method 365.2..

van Gestel, C.A.M., van der Waarde, J.J., Derksen, J.G.M., van der Hoek, E.E., Veul, M.F.X.W., Bouwens, S., Rusch, B., Kronenburg, R., Stokman, G.N.M., 2001. The use of acute and chronic bioassays to determine the ecological risk and bioremediation efficiency of oil-polluted soils. Environmental Toxicology and Chemistry 20, 1438-1449.

12. CONCLUSÕES GERAIS

O presente trabalho incluiu um estudo abrangente sobre a potencial reabilitação de dois tipos de escombreiras, uma rica em sulfuretos e outra de materiais de *gossan*, da mina de São Domingos com recurso a resíduos orgânicos e inorgânicos de baixo valor e a espécies autóctones e tolerantes mas com valor económico, *Cistus ladanifer* e *Lavandula pedunculata*. Deste modo, através do trabalho desenvolvido foi possível concluir que:

a. Os solos da mina de São Domingos como de outras áreas mineiras da Faixa Piritosa Ibérica Portuguesa (ex. Brancanes, Caveira, Chança, Lousal e Neves Corvo) apresentam uma grande heterogeneidade das suas características químicas em especial das concentrações totais de elementos potencialmente tóxicos (EPTs). Independentemente das elevadas concentrações totais de EPTs (ex. As, Ba, Cu, Pb, Sb e Zn), da baixa fertilidade e baixo valor do pH dos solos bem como da variabilidade intra- e inter-populacional observada, as plantas de *C. ladanifer* e *L. pedunculata* crescem sem sinais visíveis de toxicidade ou deficiência nutricional apresentando características adequadas para o seu uso em programas de fitoestabilização. Estas características traduzem-se por intensa absorção de EPTs, mas retenção dos mesmos nas raízes, baixa translocação dos EPTs para a parte aérea, comportamento de plantas não acumuladoras, concentrações na parte aérea abaixo do máximo permitido para consumo por animais domésticos e sistema radicular denso. Além disso, as concentrações dos EPTs nas plantas de ambas as espécies não afectam significativamente as concentrações dos nutrientes.

b. A adaptabilidade destas espécies às condições extremas da área mineira de São Domingos relaciona-se com a existência de eficientes mecanismos de tolerância ao nível fisiológico e, possivelmente, a valores de concentrações geradoras de fitotoxicidade mais elevadas (comparativamente a populações que crescem em áreas não contaminadas) contribuindo assim para ecótipos mais resistentes. Em ambas as espécies, o primeiro mecanismo de tolerância aos EPTs do solo é a sua acumulação na raiz e a redução da translocação destes para locais fotossinteticamente activos. Na *L. pedunculata*, apesar de alguns EPTs (As, Mn e Zn) atingirem concentrações consideradas fitotóxicas na parte aérea, componentes fisiológicos importantes para o normal funcionamento das plantas (ex. concentração total de proteína ou pigmentos) não são afectados negativamente, não sendo desencadeados mecanismos de tolerância, enzimáticos e não enzimáticos, relacionadas com o stresse oxidativo.

c. Os produtos derivados das plantas crescendo na área mineira (ex. chá/infusão de *L. pedunculata* e bioextractos de ambas as espécies) apresentam viabilidade económica para diferentes usos (farmacêutica, cosmética/fragância ou alimentar) devido aos EPTs não alterarem a sua qualidade (comparativamente aos mesmos produtos obtidos a partir de plantas colhidas em áreas não contaminadas). De facto, a infusão de rosmaninho oriundo da área mineira de São Domingos apresenta baixas concentrações de EPTs ou mesmo dentro da gama dos valores obtidos em outros chás/infusões, o que demonstra o baixo risco do seu consumo para a saúde humana. Ao nível dos bioextractos vegetais, estes contêm vários compostos medicinais ou odoríferos com valor económico. Deste modo, o uso estas espécies em programas de fitoestabilização de solos/escombreiras contaminados pode contribuir para um retorno económico significativo através da exploração e

comercialização destes produtos, reduzindo os gastos de implementação/manutenção do processo de reabilitação da área mineira.

d. As escombreiras de *gossan* e ricas em sulfuretos da área mineira de São Domingos apresentam significativo impacto ambiental devido, principalmente, aos seus grandes volumes e elevadas concentrações de EPTs que podem lixiviar. As escombreiras ricas em sulfuretos, devido à elevada reactividade e altas concentrações totais de EPTs, lixiviam soluções aquosas extremamente ácidas e com maiores concentrações de elementos químicos (avaliados através de lixiviados simulados e de percolação) do que as escombreiras de *gossan*. Deste modo, torna-se imprescindível a recuperação destes materiais.

e. A concepção de Tecnossolos a partir de materiais ricos em sulfuretos e misturas de resíduos orgânicos e inorgânicos não foi uma solução eficaz para a recuperação deste tipo de escombreira. De facto, apesar da melhoria da qualidade dos lixiviados destes Tecnossolos devido, especialmente, à diminuição significativa da dispersão de alguns elementos químicos (ex. As, Cu, Fe, Pb e Zn) e de SO_4, esta metodologia não foi suficiente para diminuir/impedir a oxidação dos sulfuretos, neutralização dos lixiviados ácidos e, consequentemente, desenvolvimento de vegetação e de outros processos biogeoquímicos (ex. actividade enzimática de microrganismos relacionados com os vários ciclos dos nutrientes).

f. Apesar de algumas características dos materiais de *gossan* (ex. capacidade de retenção de água, estrutura ou concentração de alguns nutrientes) poderem ser melhoradas apenas com a aplicação de alguns resíduos inorgânicos (polímeros hidrofílicos de poliacrilato), estimulando a colonização espontânea de vegetação herbácea, o aumento da fertilidade demonstrou ser um factor essencial para o desenvolvimento das espécies arbustivas estudadas. Assim, a concepção de Tecnossolos através da aplicação de misturas dos resíduos orgânicos e inorgânicos, principalmente a 75 e 150 Mg/ha, aos materiais de *gossan* foi uma solução eficiente na melhoria das características físicas, químicas e biológicas deste tipo de resíduo de mina e, consequentemente, para acelerar o processo de fitoestabilização com *C. ladanifer* e *L. pedunculata*. Além disso, a utilização destes Tecnossolos no isolamento dos resíduos ricos em sulfureto foi uma abordagem promissora permitindo, consequentemente, o estabelecimento das plantas e redução da lixiviação dos elementos químicos.

g. Assim, o sistema de reabilitação combinada das escombreiras em estudo demonstrou ser eficiente e sustentável a longo prazo, permitindo a reabilitação dos resíduos ricos em sulfuretos, estes que não são naturalmente colonizados por vegetação, que estão sujeitos a fortes processos erosivos e são geradores de drenagem ácida. A inclusão, neste sistema, de uma barreira alcalina composta por resíduos inorgânicos carbonatados (brita de rocha calcária + cinzas de biomassa) permitiu estabilizar os resíduos de sulfureto diminuindo a sua oxidação e a ascensão de soluções ácidas ricas em elementos químicos. Além disso, a concepção de Tecnossolos foi crucial para a implementação da fitoestabilização com *C. ladanifer* e *L. pedunculata* nos resíduos de *gossan*. Estas espécies de plantas apresentaram bom desenvolvimento, completando todo o ciclo fenológico, bem como comportamentos ecológicos (absorção, translocação e de acumulação de elementos químicos) semelhantes aos observados em ambiente natural e adequados à sua utilização neste sistema de

reabilitação. A elevada produção de biomassa das duas espécies poderá contribuir, consequentemente, para um retorno económico devido ao seu potencial de uso em vários sectores (farmacêutica, cosmética/fragância ou alimentar).

Apesar dos resultados promissores que foram obtidos, como trabalho futuro sugere-se a implementação do sistema de reabilitação combinada no campo a fim de confirmar a sua eficácia em condições de clima mediterrânico. Além disso, é essencial confirmar as concentrações dos EPTs nos resíduos vegetais após obtenção dos bioextractos e ajustar a metodologia de extracção dos mesmos, de modo a obter maiores teores de compostos de valor acrescentado.